

*Application
for
United States Letters Patent*

To all whom it may concern:

Be it known that **John A. Salon, et al.**

have invented certain new and useful improvements in

**DNA ENCODING A HUMAN MELANIN CONCENTRATING HORMONE RECEPTOR (MCH1) AND
USES THEREOF**

of which the following is a full, clear and exact description.

DNA ENCODING A HUMAN MELANIN CONCENTRATING HORMONE
RECEPTOR (MCH1) AND USES THEREOF

BACKGROUND OF THE INVENTION

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This application is a continuation-in-part of U.S. Serial No. 09/610,635, filed July 5, 2000, which is a continuation-in-part of PCT International Application No. PCT/US99/31169, filed December 30, 1999, which is a continuation-in-part of U.S. Serial No. 09/224,426, filed December 31, 1998, the contents of which are hereby incorporated by reference into the subject application.

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Throughout this application, various publications are referenced in parentheses by author and year. Full citations for these references may be found at the end of the specification immediately preceding the sequence listings and the claims. The disclosure of these publications in their entireties are hereby incorporated by reference into this application to describe more fully the state of the art to which this invention pertains.

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Neuroregulators comprise a diverse group of natural products that subserve or modulate communication in the nervous system. They include, but are not limited to, neuropeptides, amino acids, biogenic amines, lipids and lipid metabolites, and other metabolic byproducts. Many of these neuroregulator substances interact with specific cell surface receptors which transduce signals from the outside to the inside of the cell. G-protein coupled receptors (GPCRs) represent a major class of cell surface receptors with which many neurotransmitters interact to mediate their effects. GPCRs are predicted to have seven membrane-spanning domains and are coupled to their

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effectors via G-proteins linking receptor activation with intracellular biochemical sequelae such as stimulation of adenylyl cyclase.

5 Melanin-concentrating hormone (MCH) is a cyclic peptide originally isolated from salmonid (teleost fish) pituitaries (Kawauchi et al., 1983). In fish the 17 amino acid peptide causes aggregation of melanin within the melanophores and inhibits the release of ACTH, acting as
10 a functional antagonist of α -MSH. Mammalian MCH (19 amino acids) is highly conserved between rat, mouse, and human, exhibiting 100% amino acid identity, but its physiological roles are less clear. MCH has been reported to participate in a variety of processes including feeding,
15 water balance, energy metabolism, general arousal/attention state, memory and cognitive functions, and psychiatric disorders (for reviews, see Baker, 1991; Baker, 1994; Nahon, 1994; Knigge et al., 1996). Its role in feeding or body weight regulation is supported by a recent *Nature* publication (Qu et al., 1996) demonstrating that MCH is overexpressed in the hypothalamus of *ob/ob* mice compared with *ob/+* mice, and that fasting further increased MCH mRNA in both obese and normal mice during
20 fasting. MCH also stimulated feeding in normal rats when injected into the lateral ventricles (Rossi et al., 1997). MCH also has been reported to functionally antagonize the behavioral effects of α -MSH (Miller et al., 1993; Gonzalez et al, 1996; Sanchez et al., 1997); in addition, stress has been shown to increase POMC mRNA levels while
25 decreasing the MCH precursor preproMCH (ppMCH) mRNA levels (Presse et al., 1992). Thus MCH may serve as an integrative neuropeptide involved in the reaction to stress, as well as in the regulation of feeding and sexual activity (Baker, 1991; Knigge et al., 1996).

The gene encoding the MCH precursor (ppMCH) has been cloned and encodes two additional peptides, neuropeptide EI (13 AA) and neuropeptide GE (19AA) (Nahon et al., 1989), which may also have biological activity. MCH peptide is synthesized primarily in hypothalamic neurons (the zona incerta and lateral hypothalamus) which project diffusely to many brain areas and to the pituitary (Bittencourt et al., 1992); NEI has also been identified in medium from explanted hypothalamic neurons (Parkes and Vale, 1993). Localization studies of the mRNA indicate that MCH is also present in the periphery (testes and GI tract; Hervieu and Nahon, 1995) but the highest concentrations are in the hypothalamus. There is also evidence for differential tissue-dependent processing of proMCH in mammals. A shorter MCH gene transcript that may result from alternate splicing was found in several brain areas and peripheral tissues, and a different peptide form was also found in the periphery (Viale et al., 1997). In humans, the gene encoding authentic MCH has been localized to chromosome 12, but two copies of a variant (truncated) gene are present on chromosome 5 (Breton et al., 1993); the functional significance, if any, of the variant is not yet known. Finally, the rat MCH gene may encode an additional putative peptide in a different reading frame (Toumaniantz et al., 1996).

Although the biological effects of MCH are believed to be mediated by specific receptors, binding sites for MCH have not been well described. A tritiated ligand ($[^3\text{H}]$ -MCH) was reported to exhibit specific binding to brain membranes but was unusable for saturation analyses, so neither affinity nor B_{\max} were determined (Drozdz and Eberle, 1995). Radioiodination of the tyrosine at position thirteen resulted in a ligand with dramatically reduced biological activity (see Drozdz and Eberle, 1995). In contrast, the

radioiodination of the MCH analogue [Phe¹³, Tyr¹⁹] -MCH was successful (Drozdz et al., 1995); the ligand retained biological activity and exhibited specific binding to a variety of cell lines including mouse melanoma (B16-F1, G4F, and G4F-7), PC12, and COS cells. In G4F-7 cells, the $K_D = 0.118\text{nM}$ and the $B_{max} \sim 1100$ sites/cell. Importantly, the binding was not inhibited by α -MSH but was weakly inhibited by rat ANF ($K_i = 116\text{ nM}$ vs. 12 nM for native MCH) (Drozdz et al., 1995). More recently specific MCH binding was reported in transformed keratinocytes (Burgaud et al., 1997) and melanoma cells (Drozdz et al., 1998), where photo-crosslinking studies suggest that the receptor is a membrane protein with an apparent molecular weight of 45-50 kDaltons, compatible with the molecular weight range of the GPCR superfamily of receptors. No radioautoradiographic studies of MCH receptor localization using this ligand have been reported as yet.

Signal transduction mechanisms for MCH receptors remain obscure. No direct evidence supporting G-protein coupling exists in mammals, but two lines of weak evidence exist in teleost fish for $G_{\alpha q}$ - and/or $G_{\alpha i}$ - type coupling: 1) indirect evidence exists for MCH acting via phospholipase C in teleost fish melanophores (phospholipase C inhibitors and protein kinase C inhibitors shift the MCH dose-response curve to the right, and TPA mimics MCH at low doses (Abrao et al., 1991)); and 2) MCH-elicited pigment aggregation in fish melanophores is associated with a reduction in basal cAMP levels, similar to that observed with norepinephrine (Svensson et al., 1991; Morishita et al., 1993). Arguing against G-protein coupling is the general structural homology of MCH with ANF, whose receptors are not in the GPCR superfamily. Recently the actions of MCH were reported to be mediated via activation of a phosphatidylinositol-3-kinase pathway which is

typical of tyrosine kinase and cytokine receptors (Qu et al., 1998); however, since multiple signaling pathways (receptor cross talk) may produce this mediator no conclusions can be reached regarding MCH signal transduction pathways in mammalian systems.

The localization and biological activities of MCH peptide suggest that the modulation of MCH receptor activity may be useful in a number of therapeutic applications. The role of MCH in feeding is the best characterized of its potential clinical uses. MCH is expressed in the lateral hypothalamus, a brain area implicated in the regulation of thirst and hunger (Grillon et al., 1997); recently orexins A and B, which are potent orexigenic agents, have been shown to have very similar localization to MCH in the lateral hypothalamus (Sakurai et al., 1998). MCH mRNA levels in this brain region are increased in rats after 24 hours of food-deprivation (Hervé and Fellman, 1997); after insulin injection, a significant increase in the abundance and staining intensity of MCH immunoreactive perikarya and fibres was observed concurrent with a significant increase in the level of MCH mRNA (Bahjaoui-Bouhaddi et al., 1994). Consistent with the ability of MCH to stimulate feeding in rats (Rossi et al., 1997) is the observation that MCH mRNA levels are upregulated in the hypothalami of obese *ob/ob* mice (Qu et al., 1996), and decreased in the hypothalami of rats treated with leptin, whose food intake and body weight gains are also decreased (Sahu, 1998). MCH appears to act as a functional antagonist of the melanocortin system in its effects on food intake and on hormone secretion within the HPA (hypothalamopituitary /adrenal axis) (Ludwig et al., 1998). Further evidence of the involvement of MCH in the regulation of feeding behavior came from studies in mice in which the gene encoding the MCH peptide has been

deleted (Shimada et al., 1998). In these mice, the genetic deficiency of MCH led to a phenotype characterized by reduced body weight, low body fat content, and increased metabolic rate. More recently, it has been shown that the overexpression of the gene encoding MCH in different strains of mice can lead to obese phenotypes with and without secondary impairment of glucose homeostasis and insulin resistance (Tritos et al., 2000).

10 Together these data suggest a role for endogenous MCH in the regulation of energy balance and response to stress, and provide a rationale for the development of specific compounds acting at MCH receptors for use in the treatment of obesity and stress-related disorders.

15 In all species studied to date, a major portion of the neurons of the MCH cell group occupies a rather constant location in those areas of the lateral hypothalamus and subthalamus where they lie and may be a part of some of the so-called "extrapyramidal" motor circuits. These involve substantial striato- and pallidofugal pathways involving the thalamus and cerebral cortex, hypothalamic areas, and reciprocal connections to subthalamic nucleus, substantia nigra, and mid-brain centers (Bittencourt et al., 1992). In their location, the MCH cell group may offer a bridge or mechanism for expressing hypothalamic visceral activity with appropriate and coordinated motor activity. Clinically it may be of some value to consider the involvement of this MCH system in movement disorders, such as Parkinson's disease and Huntingdon's Chorea in which extrapyramidal circuits are known to be involved.

30 Human genetic linkage studies have located authentic hMCH loci on chromosome 12 (12q23-24) and the variant hMCH loci on chromosome 5 (5q12-13) (Pedeutour et al., 1994). Locus

12q23-24 coincides with a locus to which autosomal dominant cerebellar ataxia type II (SCA2) has been mapped (Auburger et al., 1992; Twells et al., 1992). This disease comprises neurodegenerative disorders, including an olivopontocerebellar atrophy. Furthermore, the gene for Darier's disease, has been mapped to locus 12q23-24 (Craddock et al., 1993). Darier's disease is characterized by abnormalities in keratinocyte adhesion and mental illnesses in some families. In view of the functional and neuroanatomical patterns of the MCH neural system in the rat and human brains, the MCH gene may represent a good candidate for SCA2 or Darier's disease. Interestingly, diseases with high social impact have been mapped to this locus. Indeed, the gene responsible for chronic or acute forms of spinal muscular atrophies has been assigned to chromosome 5q12-13 using genetic linkage analysis (Melki et al., 1990; Westbrook et al., 1992). Furthermore, independent lines of evidence support the assignment of a major schizophrenia locus to chromosome 5q11.2-13.3 (Sherrington et al., 1988; Bassett et al., 1988; Gilliam et al., 1989). The above studies suggest that MCH may play a role in neurodegenerative diseases and disorders of emotion.

25 Additional therapeutic applications for MCH-related compounds are suggested by the observed effects of MCH in other biological systems. For example, MCH may regulate reproductive functions in male and female rats. MCH transcripts and MCH peptide were found within germ cells in testes of adult rats, suggesting that MCH may participate in stem cell renewal and/or differentiation of early spermatocytes (Hervieu et al., 1996). MCH injected directly into the medial preoptic area (MPOA) or ventromedial nucleus (VMN) stimulated sexual activity in female rats (Gonzalez et al., 1996). In ovariectomized

rats primed with estradiol, MCH stimulated luteinizing hormone (LH) release while anti-MCH antiserum inhibited LH release (Gonzalez et al., 1997). The zona incerta, which contains a large population of MCH cell bodies, has previously been identified as a regulatory site for the pre-ovulatory LH surge (MacKenzie et al., 1984). MCH has been reported to influence release of pituitary hormones including ACTH and oxytocin. MCH analogues may also be useful in treating epilepsy. In the PTZ seizure model, injection of MCH prior to seizure induction prevented seizure activity in both rats and guinea pigs, suggesting that MCH-containing neurons may participate in the neural circuitry underlying PTZ-induced seizure (Knigge and Wagner, 1997). MCH has also been observed to affect behavioral correlates of cognitive functions. MCH treatment hastened extinction of the passive avoidance response in rats (McBride et al., 1994), raising the possibility that MCH receptor antagonists may be beneficial for memory storage and/or retention. A possible role for MCH in the modulation or perception of pain is supported by the dense innervation of the periaqueductal grey (PAG) by MCH-positive fibers. Finally, MCH may participate in the regulation of fluid intake. ICV infusion of MCH in conscious sheep produced diuretic, natriuretic, and kaliuretic changes in response to increased plasma volume (Parkes, 1996). Together with anatomical data reporting the presence of MCH in fluid regulatory areas of the brain, the results indicate that MCH may be an important peptide involved in the central control of fluid homeostasis in mammals.

In light of the localization of MCH1 throughout limbic regions of the rat CNS as described hereinafter, a series of *in vivo* behavioral experiments were carried out to evaluate the antidepressant and anxiolytic properties of

a selective MCH1 receptor antagonist. The rat Forced Swim Test and the rat Social Interaction Test were employed to evaluate the use of selective MCH1 receptor antagonists to treat depression and anxiety. These models are considered by experts in the field to reflect the potential of agents to treat depression and anxiety.

Rat Forced Swim Test (FST)

The rat Forced Swim Test (FST) is a behavioral test that is used to screen compounds for antidepressant efficacy (Porsolt et al., 1977, 1978; Porsolt, 1981). This test is widely used as it is reliable across laboratories, relatively easy to perform and is sensitive to the effects of some of the major classes of antidepressants drugs, including TCAs and MAOIs, and various atypical antidepressants. Furthermore, this test is relatively selective for antidepressant drugs, as few psychoactive drugs produce similar behavioral actions in the FST.

In the rat FST, animals are placed in a cylinder of water, from which there is no escape, for an extended period of time. Typically, animals will display a range of behaviors such as immobility, climbing, swimming, and diving, with immobility being predominant after several minutes of immersion in the water. Consequently, many past studies have only measured or scored immobility after the administration of the test agent. Unfortunately, this method does not score any other active behaviors that may be produced by potential antidepressants. Thus, if a particular class of antidepressant were to have very little effect on immobility, yet produce characteristic behaviors during the FST, these behaviors would not be scored and the conclusion would be that the compound in question does not possess antidepressant action.

Recently, however, a sampling technique was developed to score active behaviors in the FST, such as swimming, climbing and diving, in addition to immobility (Detke, et al., 1995; Lucki, 1997; Page, et al., 1999; Reneric and Lucki, 1998). This modified sampling technique has indicated that SSRIs, such as fluoxetine, paroxetine and sertraline, significantly decrease immobility and increase swimming time (Detke, et al., 1995; Page, et al., 1999). In contrast, selective reuptake inhibitors of norepinephrine (NE) increase climbing behavior but do not alter swimming time (Detke, et al., 1995; Page, et al., 1999).

Rat Social Interaction Test (SIT)

There are a number of paradigms that have been used to determine whether a compound possesses anxiolytic action. A number of these tests involve food or water deprivation, punishment or measurement of consummatory behavior (see File, et al., 1980, File, 1985, Rodgers, et al., 1997 and Treit, 1985, for review). In addition, in these models, prior conditioning reduces the uncertainty or anxiety. In general, these tests lack ethological validity.

One model that is based upon an unconditioned response that does not involve punishment or deprivation is the Social Interaction Test (SIT) (File and Hyde, 1978, 1979). In this model, rats previously housed singly are placed in a familiar, dimly lit, test arena with weight-matched, novel partners. The principal anxiogenic stimulus under these conditions is the partner novelty, which involves an unconditioned response to a potential threat. After pharmacological treatments, the following behaviors are scored as active social interaction: grooming, sniffing, biting, boxing, wrestling, following, crawling over and crawling under. A wide range of psychoactive drugs have

been examined in this paradigm and it has been shown that the social interaction test can distinguish anxiolytics from antidepressants, antipsychotics, analeptics and sedative agents (File, 1985; Guy and Gardner, 1985). This
5 test can detect anxiolytic agents such as the benzodiazepines (File and Hyde, 1978; File and Hyde, 1979; File, 1980), in addition to non-benzodiazepines, including paroxetine and other SSRIs (Lightowler, et al., 1994). Finally, the social interaction test can detect anxiogenic
10 agents, including the inverse benzodiazepine receptor agonists (File, et al., 1982, File and Pellow, 1983; File and Pellow, 1984, File, 1985).

From the binding and functional activity information described hereinafter, it has been unexpectedly discovered
15 that compounds which are MCH1 receptor antagonists are effective in animal models of obesity, depression and anxiety, which are predictive of efficacy in humans. Thus, we demonstrate that MCH1 receptor antagonists provide a novel method to treat obesity. Additionally, we
20 demonstrate that MCH1 receptor antagonists provide a novel method to treat depression and/or anxiety.

SUMMARY OF THE INVENTION

This invention provides an isolated nucleic acid encoding a human MCH1 receptor or a mutant of such human MCH1 receptor which is activated by MCH or an analog or homolog thereof.

This invention provides a nucleic acid encoding a human MCH1 receptor, wherein the nucleic acid (a) hybridizes to a nucleic acid having the defined sequence shown in Figure 1 (SEQ ID NO: 1) under low stringency conditions or a sequence complementary thereto and (b) is further characterized by its ability to cause a change in the pH of a culture of CHO cells when an MCH1 ligand is added to the culture and the CHO cells contain the nucleic acid which hybridized to the nucleic acid having the defined sequence or its complement.

This invention provides a purified human MCH1 receptor protein.

This invention provides a vector comprising a nucleic acid encoding a human MCH1 receptor, particularly a vector adapted for expression of the human MCH1 receptor in mammalian or non-mammalian cells. One such vector is a plasmid designated pEXJ.HR-TL231 (ATCC Accession No. 203197) which comprises a nucleotide sequence encoding a human MCH1 receptor.

This invention also provides a cell comprising a vector which comprises a nucleic acid encoding a human MCH1 receptor as well as a membrane preparation isolated from such cells.

This invention further provides a nucleic acid probe

comprising at least 15 nucleotides which specifically hybridizes with a nucleic acid encoding a mammalian MCH1 receptor, wherein the probe has a unique sequence corresponding to a sequence present within the nucleic acid which encodes the human MCH1 receptor or its complement, both of which are present in plasmid pEXJ.HR-TL231 (ATCC Accession No. 203197).
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This invention further provides a nucleic acid probe
10 comprising at least 15 nucleotides which specifically hybridizes with a nucleic acid encoding a mammalian MCH1 receptor, wherein the probe has a unique sequence corresponding to a sequence present within (a) the nucleic acid sequence shown in Figure 1 (SEQ ID NO: 1) or (b) the reverse complement thereof.
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This invention also provides an antisense oligonucleotide having a sequence capable of specifically hybridizing an RNA encoding a human MCH1 receptor, so as to prevent
20 translation of the RNA and an antisense oligonucleotide having a sequence capable of specifically hybridizing to the genomic DNA encoding a human MCH1 receptor.

This invention further provides an antibody capable of binding to a human MCH1 receptor as well as an agent capable of competitively inhibiting the binding of the antibody to a human MCH1 receptor.
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This invention provides a pharmaceutical composition comprising (a) an amount of the oligonucleotide described above capable of passing through a cell membrane and effective to reduce expression of a human MCH1 receptor and (b) a pharmaceutically acceptable carrier capable of passing through the cell membrane.
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Moreover, this invention provides a transgenic, nonhuman mammal expressing DNA encoding a human MCH1 receptor. This invention also provides a transgenic, nonhuman mammal comprising a homologous recombination knockout of the native human MCH1 receptor. This invention further provides a transgenic, nonhuman mammal whose genome comprises antisense DNA complementary to the DNA encoding a human MCH1 receptor so placed within the genome as to be transcribed into antisense mRNA which is complementary to mRNA encoding the human MCH1 receptor and which hybridizes to mRNA encoding the human MCH1 receptor, thereby reducing its translation.

In one embodiment this invention provides a process for identifying a chemical compound which specifically binds to a mammalian MCH1 receptor which comprises contacting cells containing DNA encoding and expressing on their cell surface a mammalian MCH1 receptor, wherein such cells do not normally express the mammalian MCH1 receptor, with the compound under conditions suitable for binding, and detecting specific binding of the chemical compound to the mammalian MCH1 receptor.

This invention provides a process for identifying a chemical compound which specifically binds to a mammalian MCH1 receptor which comprises contacting a membrane preparation from cells transfected with DNA encoding and expressing on their cell surface the mammalian MCH1 receptor, wherein such cells do not normally express the mammalian MCH1 receptor, with the compound under conditions suitable for binding, and detecting specific binding of the chemical compound to the mammalian MCH1 receptor.

This invention provides a process involving competitive

binding for identifying a chemical compound which specifically binds to a mammalian MCH1 receptor which comprises separately contacting cells expressing on their cell surface the mammalian MCH1 receptor, wherein such cells do not normally express the mammalian MCH1 receptor, with both the chemical compound and a second chemical compound known to bind to the receptor, and with only the second chemical compound, under conditions suitable for binding of both compounds, and detecting specific binding of the chemical compound to the mammalian MCH1 receptor, a decrease in the binding of the second chemical compound to the mammalian MCH1 receptor in the presence of the chemical compound indicating that the chemical compound binds to the mammalian MCH1 receptor.

This invention provides a process involving competitive binding for identifying a chemical compound which specifically binds to a mammalian MCH1 receptor which comprises separately contacting a membrane fraction from a cell extract of cells expressing on their cell surface the mammalian MCH1 receptor, wherein such cells do not normally express the mammalian MCH1 receptor, with both the chemical compound and a second chemical compound known to bind to the receptor, and with only the second chemical compound, under conditions suitable for binding of both compounds, and detecting specific binding of the chemical compound to the mammalian MCH1 receptor, a decrease in the binding of the second chemical compound to the mammalian MCH1 receptor in the presence of the chemical compound indicating that the chemical compound binds to the mammalian MCH1 receptor.

This invention provides a method of screening a plurality of chemical compounds not known to bind to a mammalian MCH1 receptor to identify a compound which specifically

5 binds to the mammalian MCH1 receptor, which comprises (a) contacting cells transfected with and expressing DNA encoding the mammalian MCH1 receptor with a compound known to bind specifically to the mammalian MCH1 receptor; (b) contacting the preparation of step (a) with the plurality of compounds not known to bind specifically to the mammalian MCH1 receptor, under conditions permitting binding of compounds known to bind the mammalian MCH1 receptor; (c) determining whether the binding of the compound known to bind to the mammalian MCH1 receptor is reduced in the presence of the compounds within the plurality of compounds, relative to the binding of the compound in the absence of the plurality of compounds; and if so (d) separately determining the binding to the mammalian MCH1 receptor of compounds included in the plurality of compounds, so as to thereby identify the compound which specifically binds to the mammalian MCH1 receptor.

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20 This invention provides a method of screening a plurality of chemical compounds not known to bind to a mammalian MCH1 receptor to identify a compound which specifically binds to the mammalian MCH1 receptor, which comprises (a) contacting a membrane preparation from cells transfected with and expressing DNA encoding a mammalian MCH1 receptor with a compound known to bind specifically to the mammalian MCH1 receptor; (b) contacting the preparation of step (a) with the plurality of compounds not known to bind specifically to the mammalian MCH1 receptor, under conditions permitting binding of compounds known to bind the mammalian MCH1 receptor; (c) determining whether the binding of the compound known to bind to the mammalian MCH1 receptor is reduced in the presence of the compounds within the plurality of compounds, relative to the binding of the compound in the absence of the plurality of compounds; and if so (d) separately determining the binding to the mammalian MCH1 receptor of compounds included in the plurality of compounds, so as to thereby identify the compound which specifically binds to the mammalian MCH1 receptor.

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compounds; and if so (d) separately determining the binding to the mammalian MCH1 receptor of compounds included in the plurality of compounds, so as to thereby identify the compound which specifically binds to the mammalian MCH1 receptor.

This invention provides a method of detecting expression of a mammalian MCH1 receptor by detecting the presence of mRNA coding for the mammalian MCH1 receptor which comprises obtaining total mRNA from the cell and contacting the mRNA so obtained with a nucleic acid probe under hybridizing conditions, detecting the presence of mRNA hybridizing to the probe, and thereby detecting the expression of the mammalian MCH1 receptor by the cell.

This invention provides a method of detecting the presence of a mammalian MCH1 receptor on the surface of a cell which comprises contacting the cell with an antibody under conditions permitting binding of the antibody to the receptor, detecting the presence of the antibody bound to the cell, and thereby detecting the presence of the mammalian MCH1 receptor on the surface of the cell.

This invention provides a method of determining the physiological effects of varying levels of activity of human MCH1 receptors which comprises producing a transgenic, nonhuman mammal whose levels of human MCH1 receptor activity are varied by use of an inducible promoter which regulates human MCH1 receptor expression.

This invention provides a method of determining the physiological effects of varying levels of activity of human MCH1 receptors which comprises producing a panel of transgenic, nonhuman mammals each expressing a different amount of human MCH1 receptor.

This invention provides a method for identifying an antagonist capable of alleviating an abnormality wherein the abnormality is alleviated by decreasing the activity of a human MCH1 receptor comprising administering a compound to the transgenic, nonhuman mammal and determining whether the compound alleviates the physical and behavioral abnormalities displayed by the transgenic, nonhuman mammal as a result of overactivity of a human MCH1 receptor, the alleviation of the abnormality identifying the compound as an antagonist. This invention also provides an antagonist identified by this method. This invention further provides a pharmaceutical composition comprising an antagonist identified by this method and a pharmaceutically acceptable carrier.

This invention provides a method of treating an abnormality in a subject wherein the abnormality is alleviated by decreasing the activity of a human MCH1 receptor which comprises administering to the subject an effective amount of this pharmaceutical composition, thereby treating the abnormality.

This invention provides a method for identifying an agonist capable of alleviating an abnormality in a subject wherein the abnormality is alleviated by increasing the activity of a human MCH1 receptor comprising administering a compound to a transgenic, nonhuman mammal, and determining whether the compound alleviates the physical and behavioral abnormalities displayed by the transgenic, nonhuman mammal, the alleviation of the abnormality identifying the compound as an agonist. This invention also provides an agonist identified by this method. This invention further provides a pharmaceutical composition comprising an agonist identified by this method and a pharmaceutically acceptable carrier. This invention

provides a method of treating an abnormality in a subject wherein the abnormality is alleviated by increasing the activity of a human MCH1 receptor which comprises administering to the subject an effective amount of this pharmaceutical composition, thereby treating the abnormality.

This invention provides a method for diagnosing a predisposition to a disorder associated with the activity of a specific mammalian allele which comprises: (a) obtaining DNA of subjects suffering from the disorder; (b) performing a restriction digest of the DNA with a panel of restriction enzymes; (c) electrophoretically separating the resulting DNA fragments on a sizing gel; (d) contacting the resulting gel with a nucleic acid probe capable of specifically hybridizing with a unique sequence included within the sequence of a nucleic acid molecule encoding a human MCH1 receptor and labeled with a detectable marker; (e) detecting labeled bands which have hybridized to the DNA encoding a human MCH1 receptor labeled with a detectable marker to create a unique band pattern specific to the DNA of subjects suffering from the disorder; (f) preparing DNA obtained for diagnosis by steps (a)-(e); and (g) comparing the unique band pattern specific to the DNA of subjects suffering from the disorder from step (e) and the DNA obtained for diagnosis from step (f) to determine whether the patterns are the same or different and to diagnose thereby predisposition to the disorder if the patterns are the same.

This invention provides a method of preparing a purified human MCH1 receptor which comprises: (a) inducing cells to express the human MCH1 receptor; (b) recovering the human MCH1 receptor from the induced cells; and (c) purifying the human MCH1 receptor so recovered.

This invention provides a method of preparing a purified human MCH1 receptor which comprises: (a) inserting nucleic acid encoding the human MCH1 receptor in a suitable vector; (b) introducing the resulting vector in a suitable host cell; (c) placing the resulting cell in suitable condition permitting the production of the isolated human MCH1 receptor; (d) recovering the human MCH1 receptor produced by the resulting cell; and (e) purifying the human MCH1 receptor so recovered.

This invention provides a process for determining whether a chemical compound is a mammalian MCH1 receptor agonist which comprises contacting cells transfected with and expressing DNA encoding the mammalian MCH1 receptor with the compound under conditions permitting the activation of the mammalian MCH1 receptor, and detecting an increase in mammalian MCH1 receptor activity, so as to thereby determine whether the compound is a mammalian MCH1 receptor agonist. This invention also provides a pharmaceutical composition which comprises an amount of a mammalian MCH1 receptor agonist determined by this process effective to increase activity of a mammalian MCH1 receptor and a pharmaceutically acceptable carrier.

This invention provides a process for determining whether a chemical compound is a mammalian MCH1 receptor antagonist which comprises contacting cells transfected with and expressing DNA encoding the mammalian MCH1 receptor with the compound in the presence of a known mammalian MCH1 receptor agonist, under conditions permitting the activation of the mammalian MCH1 receptor, and detecting a decrease in mammalian MCH1 receptor activity, so as to thereby determine whether the compound is a mammalian MCH1 receptor antagonist. This invention also provides a pharmaceutical composition which comprises

an amount of a mammalian MCH1 receptor antagonist determined by this process effective to reduce activity of a mammalian MCH1 receptor and a pharmaceutically acceptable carrier.

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This invention provides a process for determining whether a chemical compound specifically binds to and activates a mammalian MCH1 receptor, which comprises contacting cells producing a second messenger response and expressing on their cell surface the mammalian MCH1 receptor, wherein such cells do not normally express the mammalian MCH1 receptor, with the chemical compound under conditions suitable for activation of the mammalian MCH1 receptor, and measuring the second messenger response in the presence and in the absence of the chemical compound, a change in the second messenger response in the presence of the chemical compound indicating that the compound activates the mammalian MCH1 receptor. This invention also provides a compound determined by this process. This invention further provides a pharmaceutical composition which comprises an amount of the compound (a MCH1 receptor agonist) determined by this process effective to increase activity of a mammalian MCH1 receptor and a pharmaceutically acceptable carrier.

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This invention provides a process for determining whether a chemical compound specifically binds to and inhibits activation of a mammalian MCH1 receptor, which comprises separately contacting cells producing a second messenger response and expressing on their cell surface the mammalian MCH1 receptor, wherein such cells do not normally express the mammalian MCH1 receptor, with both the chemical compound and a second chemical compound known to activate the mammalian MCH1 receptor, and with only the second chemical compound, under conditions suitable for

P R I V A T E C O M M U N I C A T I O N S

activation of the mammalian MCH1 receptor, and measuring the second messenger response in the presence of only the second chemical compound and in the presence of both the second chemical compound and the chemical compound, a smaller change in the second messenger response in the presence of both the chemical compound and the second chemical compound than in the presence of only the second chemical compound indicating that the chemical compound inhibits activation of the mammalian MCH1 receptor. This invention also provides a compound determined by this process. This invention further provides a pharmaceutical composition which comprises an amount of the compound (a mammalian MCH1 receptor antagonist) determined by this effective to reduce activity of a mammalian MCH1 receptor and a pharmaceutically acceptable carrier.

This invention provides a method of screening a plurality of chemical compounds not known to activate a mammalian MCH1 receptor to identify a compound which activates the mammalian MCH1 receptor which comprises: (a) contacting cells transfected with and expressing the mammalian MCH1 receptor with the plurality of compounds not known to activate the mammalian MCH1 receptor, under conditions permitting activation of the mammalian MCH1 receptor; (b) determining whether the activity of the mammalian MCH1 receptor is increased in the presence of the compounds; and if so (c) separately determining whether the activation of the mammalian MCH1 receptor is increased by each compound included in the plurality of compounds, so as to thereby identify the compound which activates the mammalian MCH1 receptor. This invention also provides a compound identified by this method. This invention further provides a pharmaceutical composition which comprises an amount of the compound (a mammalian MCH1 receptor agonist) identified by this method effective to

increase activity of a mammalian MCH1 receptor and a pharmaceutically acceptable carrier.

This invention provides a method of screening a plurality of chemical compounds not known to inhibit the activation of a mammalian MCH1 receptor to identify a compound which inhibits the activation of the mammalian MCH1 receptor, which comprises: (a) contacting cells transfected with and expressing the mammalian MCH1 receptor with the plurality of compounds in the presence of a known mammalian MCH1 receptor agonist, under conditions permitting activation of the mammalian MCH1 receptor; (b) determining whether the activation of the mammalian MCH1 receptor is reduced in the presence of the plurality of compounds, relative to the activation of the mammalian MCH1 receptor in the absence of the plurality of compounds; and if so (c) separately determining the inhibition of activation of the mammalian MCH1 receptor for each compound included in the plurality of compounds, so as to thereby identify the compound which inhibits the activation of the mammalian MCH1 receptor. This invention also provides a compound identified by this method. This invention further provides a pharmaceutical composition which comprises an amount of the compound (a mammalian MCH1 receptor antagonist) identified by this process effective to decrease activity of a mammalian MCH1 receptor and a pharmaceutically acceptable carrier.

This invention provides a method of treating an abnormality in a subject wherein the abnormality is alleviated by increasing the activity of a mammalian MCH1 receptor which comprises administering to the subject an amount of a compound which is a mammalian MCH1 receptor agonist effective to treat the abnormality.

This invention provides a method of treating an abnormality in a subject wherein the abnormality is alleviated by decreasing the activity of a mammalian MCH1 receptor which comprises administering to the subject an amount of a compound which is a mammalian MCH1 receptor antagonist effective to treat the abnormality.

This invention provides a process for making a composition of matter which specifically binds to a mammalian MCH1 receptor which comprises identifying a chemical compound using any of the processes described herein for identifying a compound which binds to and/or activates or inhibits activation of a mammalian MCH1 receptor and then synthesizing the chemical compound or a novel structural and functional analog or homolog thereof. This invention further provides a process for preparing a pharmaceutical composition which comprises administering a pharmaceutically acceptable carrier and a pharmaceutically acceptable amount of a chemical compound identified by any of the processes described herein for identifying a compound which binds to and/or activates or inhibits activation of a mammalian MCH1 receptor or a novel structural and functional analog or homolog thereof.

This invention provides a process for determining whether a chemical compound is a human MCH1 receptor antagonist which comprises contacting cells transfected with and expressing DNA encoding the human MCH1 receptor with the compound in the presence of a known human MCH1 receptor agonist, under conditions permitting the activation of the human MCH1 receptor, and detecting a decrease in human MCH1 receptor activity, so as to thereby determine whether the compound is a human MCH1 receptor antagonist, wherein the DNA encoding the human MCH1 receptor comprises the sequence shown in Figure 1 (Seq. ID No. 1) or contained in

plasmid pEXJ.HR-TL231 (ATCC Accession No. 203197), the known human MCH1 receptor agonist is MCH or a homolog or analog of MCH, and the cells do not express the MCH1 receptor prior to transfecting them.

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This invention also provides a process for determining whether a chemical compound specifically binds to and inhibits activation of a human MCH1 receptor, which comprises separately contacting cells expressing on their cell surface the human MCH1 receptor and producing a second messenger response upon activation of the human MCH1 receptor, wherein such cells do not normally express the human MCH1 receptor and the DNA encoding the human MCH1 receptor comprises the sequence shown in Figure 1 (Seq. ID No. 1) or contained in plasmid pEXJ.HR-TL231 (ATCC Accession No. 203197), with both the chemical compound and a second chemical compound known to activate the human MCH1 receptor, and with only the second chemical compound, under conditions suitable for activation of the human MCH1 receptor, and measuring the second messenger response in the presence of only the second chemical compound and in the presence of both the second chemical compound and the chemical compound, a smaller change in the second messenger response in the presence of both the chemical compound and the second chemical compound than in the presence of only the second chemical compound indicating that the chemical compound inhibits activation of the human MCH1 receptor, wherein the second chemical compound is MCH or a homolog or analog of MCH.

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30 This invention further provides a method of screening a plurality of chemical compounds not known to inhibit the activation of a human MCH1 receptor to identify a compound which inhibits the activation of the human MCH1 receptor, which comprises:

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- 5 (a) contacting cells transfected with and expressing the human MCH1 receptor, wherein such cells do not normally express the human MCH1 receptor and the DNA encoding the human MCH1 receptor comprises the sequence shown in Figure 1 (Seq. ID No. 1) or contained in plasmid pEXJ.HR-TL231 (ATCC Accession No. 203197), with the plurality of compounds in the presence of a known human MCH1 receptor agonist, under conditions permitting activation of the human MCH1 receptor, wherein the known MCH1 receptor agonist is MCH or a homolog or analog of MCH;

10 (b) determining whether the activation of the human MCH1 receptor is reduced in the presence of the plurality of compounds, relative to the activation of the human MCH1 receptor in the absence of the plurality of compounds; and if so

15 (c) separately determining the extent of inhibition of activation of the human MCH1 receptor for each compound included in the plurality of compounds, so as to thereby identify the compound which inhibits the activation of the human MCH1 receptor.

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25 This invention provides a process for making a composition of matter which specifically binds to a human MCH1 receptor which comprises identifying a chemical compound which specifically binds to the human MCH1 receptor and then synthesizing the chemical compound or a structural and functional analog or homolog thereof, wherein the chemical compound is identified as binding to the human MCH1 receptor by a process involving competitive binding which comprises contacting cells expressing on their cell surface the human MCH1 receptor, with both the chemical compound and a second chemical compound known to bind to

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the receptor, and separately with only the second chemical compound, under conditions suitable for binding of both compounds, and detecting the extent of specific binding of the chemical compound to the human MCH1 receptor, a
5 decrease in the binding of the second chemical compound to the human MCH1 receptor in the presence of the chemical compound indicating that the chemical compound binds to the human MCH1 receptor, wherein the cells do not normally express the human MCH1 receptor, the human MCH1 receptor is encoded by nucleic acid comprising the sequence shown
10 in Figure 1 (Seq. ID No. 1) or contained in plasmid pEXJ.HR-TL231 (ATCC Accession No. 203197), and the second chemical compound is MCH or a homolog or analog of MCH.

15 This invention further provides a process for making a composition of matter which specifically binds to a human MCH1 receptor which comprises identifying a chemical compound which specifically binds to the human MCH1 receptor and then synthesizing the chemical compound or a structural and functional analog or homolog thereof,
20 wherein the chemical compound is identified as binding to the human MCH1 receptor by a process involving competitive binding which comprises contacting a membrane preparation from cells expressing on their cell surface the human MCH1 receptor, with both the chemical compound and a second chemical compound known to bind to the receptor, and separately with only the second chemical compound, under conditions suitable for binding of both compounds, and detecting the extent of specific binding of the chemical compound to the human MCH1 receptor, a decrease in the
25 binding of the second chemical compound to the human MCH1 receptor in the presence of the chemical compound indicating that the chemical compound binds to the human MCH1 receptor, wherein the cells do not normally express the human MCH1 receptor, the human MCH1 receptor is
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encoded by nucleic acid comprising the sequence shown in Figure 1 (Seq. ID No. 1) or contained in plasmid pEXJ.HR-TL231 (ATCC Accession No. 203197), and the second chemical compound is MCH or a homolog or analog of MCH.

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This invention also provides a process for making a composition of matter which is a human MCH1 receptor antagonist which comprises identifying a chemical compound which is a human MCH1 receptor antagonist and then synthesizing the chemical compound or a structural and functional analog or homolog thereof, wherein the chemical compound is identified as a human MCH1 receptor antagonist by a process which comprises contacting cells transfected with and expressing DNA encoding the human MCH1 receptor with the compound in the presence of a known human MCH1 receptor agonist, under conditions permitting the activation of the human MCH1 receptor, and detecting a decrease in human MCH1 receptor activity, so as to thereby determine whether the compound is a human MCH1 receptor antagonist, wherein the cells do not normally express the human MCH1 receptor, the human MCH1 receptor is encoded by nucleic acid comprising the sequence shown in Figure 1 (Seq. ID No. 1) or contained in plasmid pEXJ.HR-TL231 (ATCC Accession No. 203197), and the known human MCH1 receptor agonist is MCH or a homolog or analog of MCH.

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This inventions still further provides a process for making a composition of matter which specifically binds to and inhibits the activation of a human MCH1 receptor which comprises identifying a chemical compound which specifically binds to and inhibits the activation of the human MCH1 receptor and then synthesizing the chemical compound or a structural and functional analog or homolog thereof, wherein the chemical compound is identified as binding to and inhibiting the activation of the human MCH1

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receptor by a process which comprises separately contacting cells expressing on their cell surface the human MCH1 receptor and producing a second messenger response upon activation of the human MCH1 receptor,
5 wherein such cells do not normally express the human MCH1 receptor and the human MCH1 receptor is encoded by nucleic acid comprising the sequence shown in Figure 1 (Seq. ID No. 1) or contained in plasmid pEXJ.HR-TL231 (ATCC Accession No. 203197), with both the chemical compound and
10 a second chemical compound known to activate the human MCH1 receptor, and with only the second chemical compound, under conditions suitable for activation of the human MCH1 receptor, and measuring the second messenger response in the presence of only the second chemical compound and in the presence of both the second chemical compound and the
15 chemical compound, a smaller change in the second messenger response in the presence of both the chemical compound and the second chemical compound than in the presence of only the second chemical compound indicating that the chemical compound inhibits activation of the
20 human MCH1 receptor, wherein the second chemical compound is MCH or a homolog or analog of MCH.

This invention provides a process for preparing a composition which comprises identifying a chemical compound which specifically binds to a human MCH1 receptor, and then admixing a carrier and the chemical compound or a structural and functional analog or homolog thereof, wherein the chemical compound is identified as binding to the human MCH1 receptor by a process involving competitive binding which comprises contacting cells expressing on their cell surface the human MCH1 receptor, with both the chemical compound and a second chemical compound known to bind to the receptor, and separately with only the second chemical compound, under conditions
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suitable for binding of both compounds, and detecting the extent of specific binding of the chemical compound to the human MCH1 receptor, a decrease in the binding of the second chemical compound to the human MCH1 receptor in the presence of the chemical compound indicating that the chemical compound binds to the human MCH1 receptor, wherein the cells do not normally express the human MCH1 receptor, the human MCH1 receptor is encoded by nucleic acid comprising the sequence shown in Figure 1 (Seq. ID No. 1) or contained in plasmid pEXJ.HR-TL231 (ATCC Accession No. 203197), and the second chemical compound is MCH or a homolog or analog of MCH.

This invention further provides a process for preparing a composition which comprises identifying a chemical compound which specifically binds to a human MCH1 receptor, and then admixing a carrier and the chemical compound or a structural and functional analog or homolog thereof, wherein the chemical compound is identified as binding to the human MCH1 receptor by a process involving competitive binding which comprises contacting a membrane preparation from cells expressing on their cell surface the human MCH1 receptor, with both the chemical compound and a second chemical compound known to bind to the receptor, and separately with only the second chemical compound, under conditions suitable for binding of both compounds, and detecting the extent of specific binding of the chemical compound to the human MCH1 receptor, a decrease in the binding of the second chemical compound to the human MCH1 receptor in the presence of the chemical compound indicating that the chemical compound binds to the human MCH1 receptor, wherein the cells do not normally express the human MCH1 receptor, the human MCH1 receptor is encoded by nucleic acid comprising the sequence shown in Figure 1 (Seq. ID No. 1) or contained in plasmid

pEXJ.HR-TL231 (ATCC Accession No. 203197), and the second chemical compound is MCH or a homolog or analog of MCH.

5 This invention also provides a process for preparing a composition which comprises identifying a chemical compound which is a human MCH1 receptor antagonist, and then admixing a carrier and the chemical compound or a structural and functional analog or homolog thereof, wherein the chemical compound is identified as a human MCH1 receptor antagonist by a process which comprises contacting cells transfected with and expressing DNA encoding the human MCH1 receptor with the compound in the presence of a known human MCH1 receptor agonist, under conditions permitting the activation of the human MCH1 receptor, and detecting a decrease in human MCH1 receptor activity, so as to thereby determine whether the compound is a human MCH1 receptor antagonist, wherein the cells do not normally express the human MCH1 receptor, the human MCH1 receptor is encoded by nucleic acid comprising the sequence shown in Figure 1 (Seq. ID No. 1) or contained in plasmid pEXJ.HR-TL231 (ATCC Accession No. 203197), and the known human MCH1 receptor agonist is MCH or a homolog or analog of MCH.

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This invention still further provides a process for preparing a composition which comprises identifying a chemical compound which specifically binds to and inhibits the activation of a human MCH1 receptor, and then admixing a carrier and the chemical compound or a structural and functional analog or homolog thereof, wherein the chemical compound is identified as binding to and inhibiting activation of the human MCH1 receptor by a process which comprises separately contacting cells expressing on their cell surface the human MCH1 receptor and producing a

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second messenger response upon activation of the human MCH1 receptor, wherein such cells do not normally express the human MCH1 receptor and the human MCH1 receptor is encoded by nucleic acid comprising the sequence shown in Figure 1 (Seq. ID No. 1) or contained in plasmid pEXJ.HR-TL231 (ATCC Accession No. 203197), with both the chemical compound and a second chemical compound known to activate the human MCH1 receptor, and with only the second chemical compound, under conditions suitable for activation of the human MCH1 receptor, and measuring the second messenger response in the presence of only the second chemical compound and in the presence of both the second chemical compound and the chemical compound, a smaller change in the second messenger response in the presence of both the chemical compound and the second chemical compound than in the presence of only the second chemical compound indicating that the chemical compound inhibits activation of the human MCH1 receptor, wherein the second chemical compound is MCH or a homolog or analog of MCH.

This invention provides a method of treating an eating disorder or obesity in a subject which comprises administering to the subject a therapeutically effective amount of an MCH1 antagonist which inhibits the activation of the MCH1 receptor.

This invention provides a method of reducing the body mass of a subject which comprises administering to the subject an amount of an MCH1 antagonist effective to reduce the body mass of the subject.

This invention further provides a method of treating an eating disorder in a subject which comprises administering to the subject a therapeutically effective amount of an MCH1 agonist which activates the MCH1 receptor.

This invention also provides a method of treating depression and/or anxiety in a subject which comprises administering to the subject a composition comprising a pharmaceutically acceptable carrier and a therapeutically effective amount of a MCH1 receptor antagonist, wherein:

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 - (a) (1) the MCH1 receptor antagonist does not inhibit the activity of central monoamine oxidase A greater than 50 percent, at a concentration of 10mM; and (2) the MCH1 receptor antagonist does not inhibit the activity of central monoamine oxidase B greater than 50 percent, at a concentration of 10mM; and
 - (b) the MCH1 receptor antagonist binds to the human MCH1 receptor with a binding affinity at least ten-fold higher than the binding affinity with which it binds to each of the following transporters: serotonin transporter, norepinephrine transporter, and dopamine transporter.

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BRIEF DESCRIPTION OF THE FIGURES

Figure 1

5 Nucleotide sequence encoding a human MCH1 receptor (MCH1) (SEQ ID NO: 1). Three potential start (ATG) codons and the stop (TGA) codon are underlined.

Figure 2

10 Dduced amino acid sequence (SEQ ID NO: 2) of the human MCH1 receptor (MCH1) encoded by the nucleotide sequence shown Figure 1 (SEQ ID NO: 1).

Figure 3

15 Dduced amino acid sequence for human MCH1 (SEQ ID NO: 2). The seven putative transmembrane (TM) regions are underlined.

Figure 4

20 Nucleotide sequence of rat MCH1 (SEQ ID NO: 3). One start (ATG) codon and the stop codon (TGA) are underlined.

Figure 5

Dduced amino acid sequence for rat MCH1 (SEQ ID NO: 4).

25 Figure 6

MCH1-mediated PI dose response to MCH.

Figure 7

MCH1 challenge with several compounds of interest.

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Figure 8

MCH1-mediated extracellular acidification response to MCH and Phe¹³,Tyr¹⁹-MCH. Results are reported as the average of two independent experiments performed in duplicate.

Figure 9

Transcriptional response of MCH1-transfected Cos-7 cells to MCH.

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Figure 10

Binding of [¹²⁵I]Phe¹³,Tyr¹⁹-MCH on MCH1-transfected Cos-7 cell membranes. Results are means ± S.E.M. (vertical lines) of triplicate determinations.

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Figure 11

RT-PCR detection of MCH1 receptor mRNA in human mRNA samples.

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Figure 12

Amino acid alignment of the N-terminal regions of MCH1 receptors encoded by various plasmids. The mutations present in R106 (SEQ ID NO: 16) and R114 (SEQ ID NO: 17) are shown in lower case. Potential initiating methionines are shown in bold. The amino acid sequence downstream of position 100 is identical for all four plasmids.

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Figure 13

Amino acid sequence (SEQ ID NO: 26) of the mutant human MCH1 receptor encoded by plasmid R106.

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Figure 14

Amino acid sequence (SEQ ID NO: 27) of the mutant human MCH1 receptor encoded by plasmid R114.

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Figure 15

Amino acid sequence (SEQ ID NO: 28) of the mutant human MCH1 receptor encoded by plasmid BO120.

Figure 16

Antagonism by Compound 10 shown by the phosphoinositide response induced by MCH in Cos-7 cells transfected with MCH1. Inset: Schild plot, y axis = $((EC50_{MCH+Cmpd10}/EC50_{MCH}) - 1)$; x axis = Log (Cmpd10) [M]. The analysis by linear regression analysis estimated a pA₂ (x-intercept) = 9.24, slope = 0.97 ± 0.2 and $r^2 = 0.94$.

Figure 17

Saturation equilibrium binding of [³H]Compound 10 to the human MCH1 receptor. Membrane preparations from Cos-7 cells transfected with MCH1 were incubated with varying concentrations of [³H]Compound 10 (SA: 56 Ci/mmol) at room temperature for 90 min, in a volume of 0.250 ml. The reaction was terminated by filtration in GF/C filters, and the radioactivity determined by scintillation counting. Non-specific binding was defined as the amount of radioactivity retained in the filter after incubating the reaction mixture in the presence of unlabeled Compound 10 (10 nM).

Figure 18

Competition binding of [³H]Compound 10 to the human MCH1 receptor. Membrane preparations from Cos-7 cells transfected with MCH1 were incubated with 0.4 nM [³H]Compound in the presence of varying concentrations of MCH (from 1E-11 to 1E-6 M) or unlabeled Compound 10 (from 1E-10 to 1E-5 M), for 90 min at room temperature. The reaction was terminated by filtration in GF/C filters and the radioactivity bound to the membrane was determined by scintillation counting.

Figure 19

Autoradiographic localization of MCH1 receptor binding

sites in the rat diencephalon. A. Total MCH1 receptor binding obtained with 0.1 nM [³H]Compound 10 in the presence of 1 μM prazosin and 100 μM dopamine. B. Nonspecific binding observed in the presence of 1 μM cold Compound 10.

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Figures 20A and 20B

Autoradiographic distribution of MCH1 binding sites using [³H]Compound 10 in the presence of 1 μM prazosin and 100 μM dopamine in the rat CNS presented rostrocaudally. Coronal rat brain sections at the level of the frontal cortex (A), the forebrain/basal ganglia (B), the basal ganglia (C), the diencephalon (D-H), the midbrain (I-J), the brain stem (K-L), and transverse through the lumbar spinal cord (M). Note the dense labeling of several brain regions such as the caudate-putamen (CPu) and accumbens nucleus (AcbSh and AcbC) (B). Moderate labeling was observed in the hippocampus (E-H), subthalamic nucleus (F) and locus coeruleus (L) while weaker labeling is seen in the thalamus and hypothalamus (D-H).

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List of Abbreviations

AAV	anterior amygdaloid area, ventral
AcbC	accumbens nucleus, core
AcbSh	accumbens nucleus, core
ACo	anterior cortical amygdaloid nucleus
AD	anterodorsal thalamic nucleus
AH	anterior hypothalamus
AI	agranular insular cortex
Arc	arcuate hypothalamic nucleus
AON	anterior olfactory nucleus
AU	auditory cortex
AV	anteroventral hypothalamic nucleus
BLA	basolateral amygdaloid nucleus

	BSTM	bed nucleus of the stria terminalis, medial div.
	CA1, 2, 3	fields CA1, 2, 3 of hippocampus
	Cg	cingulate cortex
5	CL	claustrum
	CPu	caudate-putamen
	DLG	dorsal lateral geniculate
	DM	dorsomedial hypothalamic nucleus
	DR	dorsal raphe nucleus
10	DTN	dorsal tegmental nucleus
	Ent	entorhinal cortex
	GP	globus pallidus
	IAM	interanteromedial thalamic nucleus
	IC	inferior colliculus
15	ICjM	islands of Calleja, major island
	IG	indusium griseum
	La	lateral amygdaloid nucleus
	LC	locus coeruleus
	LD	laterodorsal thalamic nucleus
20	LH	lateral hypothalamic area
	LO	lateral preoptic area
	LSD	lateral septal nucleus, dorsal part
	LSO	lateral superior olive
	M1	primary motor cortex
25	Me	medial amygdaloid nucleus
	MG	medial geniculate nucleus
	MHb	medial habenular nucleus
	MM	medial mammillary nucleus
	MPO	medial preoptic area
30	OC	occipital cortex
	PAG	periaqueductal gray
	PB	parabrachial nucleus
	PF	parafascicular thalamic nucleus
	PH	posterior hypothalamic area
35	Pir	piriform cortex

	PMCo	posteromedial amygdaloid nucleus
	Pn	pontine nuclei
	Po	posterior thalamic nuclear group
	PVA	paraventricular thalamic nucleus
5	PVP	paraventricular thalamic nucleus, posterior
	RSG	retrosplenial granular cortex
	SC	superior colliculus
	SNR	substantia nigra, reticular part
	STh	subthalamic nucleus
10	S1	primary somatosensory cortex
	so	stratum oriens field CA1
	sr	stratum radiatum field CA1
	Tu	olfactory tubercle
	V2	secondary visual cortex
15	VL	ventrolateral thalamic nucleus
	VMH	ventromedial hypothalamic nucleus
	VP	ventroposterior thalamic nucleus

Figure 21

Effect of Compound 10 on MCH-induced stimulation of food intake in rats. MCH (3 nmol) or vehicle was administered into the third ventricle, and food intake measured 30, 60 and 120 minutes later. Some rats were pretreated with vehicle or Compound 10 (1 or 10 mg/kg) i.p. 20 minutes prior to i.c.v. injection.

* Significantly greater than vehicle, + significantly less than vehicle /MCH.

Figure 22

Effect of Compound 10 on body weight gain in young growing rats. Compound 10 (10 mg/kg/day), fenfluramine (6 mg/kg/day) or vehicle were administered to rats for 14 days via subcutaneously implanted osmotic minipumps. Significant differences from vehicle are denoted by **P<0.001, *P<0.01, xP<0.05, as determined by ANOVA and

Newman-Keuls test.

Figure 23

Effect of Compound 10 on body weight gain in young growing rats. Compound 10 (1, 3 or 10 mg/kg) or vehicle (dashed line) was administered to rats twice daily by i.p. injection. Significant differences from vehicle are denoted by **P<0.001, *P<0.01, as determined by ANOVA and Newman-Keuls test.

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Figure 24

Effect of Compound 94 on body weight gain in young growing rats. Compound 94 (3, 10 or 30 mg/kg) or vehicle was administered to rats twice daily by i.p. injection. Significant differences from vehicle are denoted by +P<0.05, *P<0.01, as determined by ANOVA and Newman-Keuls test.

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Figure 25

Effect of Compound 95 on body weight gain in young growing rats. Compound 67173 (3, 10 or 30 mg/kg) or vehicle was administered to rats twice daily by i.p. injection. Significant differences from vehicle are denoted by *P<0.001, as determined by ANOVA and Newman-Keuls test.

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Figure 26

Effect of Compound 10 on sweetened condensed milk consumption in rats. Rats were trained to drink sweetened condensed milk for 20 minutes a day. On the test day, Compound 10 (3, 10 or 30 mg/kg), fenfluramine (3 mg/kg) or vehicle was administered i.p. 30 minutes prior to milk exposure. Significant differences from vehicle are denoted by +P<0.05, **P<0.001 as determined by two-tailed t-test.

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DETAILED DESCRIPTION OF THE INVENTION

Throughout this application, the following standard abbreviations are used to indicate specific nucleotide bases:

	A = adenine
	G = guanine
	C = cytosine
	T = thymine
5	U = uracil
	M = adenine or cytosine
	R = adenine or guanine
	W = adenine, thymine, or uracil
	S = cytosine or guanine
10	Y = cytosine, thymine, or uracil
	K = guanine, thymine, or uracil
	V = adenine, cytosine, or guanine (not thymine or uracil)
15	H = adenine, cytosine, thymine, or uracil (not guanine)
	D = adenine, guanine, thymine, or uracil (not cytosine)
	B = cytosine, guanine, thymine, or uracil (not adenine)
20	N = adenine, cytosine, guanine, thymine, or uracil (or other modified base such as inosine)
	I = inosine

Furthermore, the term "agonist" is used throughout this application to indicate any peptide or non-peptidyl compound which increases the activity of any of the polypeptides of the subject invention. The term "antagonist" is used throughout this application to indicate any peptide or non-peptidyl compound which decreases the activity of any of the polypeptides of the

subject invention. The term "mammalian" is used throughout this invention to include mutant forms of the human MCH1 receptor.

5 The activity of a G-protein coupled receptor such as the polypeptides disclosed herein may be measured using any of a variety of functional assays in which activation of the receptor in question results in an observable change in the level of some second messenger system, including, but not limited to, adenylyl cyclase, calcium mobilization, arachidonic acid release, ion channel activity, inositol phospholipid hydrolysis or guanylyl cyclase. Heterologous expression systems utilizing appropriate host cells to express the nucleic acid of the subject invention are used 10 to obtain the desired second messenger coupling. Receptor activity may also be assayed in an oocyte expression system.
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In the case that a receptor has activity in the absence of 20 an agonist (constitutive receptor activity) the antagonist may act as an inverse agonist or an allosteric modulator, as opposed to a neutral antagonist, and suppress receptor signaling independent of the agonist (Lutz and Kenakin, 1999). The categories of "antagonist compounds" are 25 therefore seen to include 1) neutral antagonists (which block agonist actions but do not affect constitutive activity); 2) inverse agonists (which block agonist actions as well as constitutive activity by stabilizing an inactive receptor conformation); 3) and allosteric modulators (which block agonist actions to a limited extent and which may also block constitutive activity through allosteric regulation). The probability that an antagonist is neutral and therefore of "zero efficacy" is 30 relatively low, given that this would require identical affinities for different tertiary conformations of the
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receptor. Thus, Kenakin proposed in 1996 that, "with the development of sensitive test systems for the detection of inverse agonism will come a reclassification of many drugs....it might be observed that numerous previously classified neutral antagonists may be inverse agonists" (Kenakin, 1996). Indeed, there is now evidence from studies with known pharmacological agents to support the existence of inverse agonists for numerous receptors, including histamine, 5HT_{1A}, 5HT_{2C}, cannabinoid, dopamine, calcitonin and human formyl peptide receptors, among others (de Ligt, et al, 2000; Herrick-Davis, et al, 2000; Bakker, et al, 2000). In the case of the 5HT_{2C} receptor, clinically effective atypical antipsychotics drugs such as sertindole, clozapine, olanzapine, ziprasidone, risperidone, zotepine, tiospirone, fluperlapine and tenilapine displayed potent inverse activity whereas typical antipsychotic drugs such as chlorpromazine, thioridazine, spiperone and thiothixene were classified as neutral antagonists (Herrick-Davis et al, 2000). In the case of the histamine H₁ receptor, the therapeutically used anti-allergics cetirizine, loratadine and epinastine were found to be inverse agonists. These findings further extend the idea that many compounds previously thought of as neutral antagonists will be reclassified as inverse agonists when tested in a constitutively active receptor system (de Ligt et al, 2000).

It is possible that the human MCH1 receptor gene contains introns and furthermore, the possibility exists that additional introns could exist in coding or non-coding regions. In addition, spliced form(s) of mRNA may encode additional amino acids either upstream of the currently defined starting methionine or within the coding region. Further, the existence and use of alternative exons is possible, whereby the mRNA may encode different amino

acids within the region comprising the exon. In addition, single amino acid substitutions may arise via the mechanism of RNA editing such that the amino acid sequence of the expressed protein is different than that encoded by the original gene. (Burns et al., 1996; Chu et al., 1996). Such variants may exhibit pharmacologic properties differing from the polypeptide encoded by the original gene.

10 This invention provides splice variants of the human MCH1 receptor disclosed herein. This invention further provides for alternate translation initiation sites and alternately spliced or edited variants of nucleic acids encoding the human MCH1 receptor of this invention.

15 The nucleic acid of the subject invention also includes nucleic acid analogs of the human MCH1 receptor gene, wherein the human MCH1 receptor gene comprises the nucleic acid sequence shown in Fig. 1 or contained in plasmid pEXJ.HR-TL231 (ATCC Accession No. 203197). Nucleic acid analogs of the human MCH1 receptor genes differ from the human MCH1 receptor gene described herein in terms of the identity or location of one or more nucleic acid bases (deletion analogs containing less than all of the nucleic acid bases shown in Fig. 1 or contained in plasmid pEXJ.HR-TL231, substitution analogs wherein one or more nucleic acid bases shown in Fig. 1 or contained in plasmids pEXJ.HR-TL231 are replaced by other nucleic acid bases, and addition analogs, wherein one or more nucleic acid bases are added to a terminal or medial portion of the nucleic acid sequence) and which encode proteins which share some or all of the properties of the proteins encoded by the nucleic acid sequences shown in Fig. 1 or contained in plasmid pEXJ.HR-TL231. In one embodiment of the present invention, the nucleic acid analog encodes a

protein which comprises an amino acid sequence as shown in Fig. 2 or encoded by the nucleic acid sequence contained in plasmid pEXJ.HR-TL231. In another embodiment, the nucleic acid analog encodes a protein comprising an amino acid sequence which differs from the amino acid sequences shown in Fig. 2 or encoded by the nucleic acid contained in plasmids pEXJ.HR-TL231. In a further embodiment, the protein encoded by the nucleic acid analog has a function which is the same as the function of the receptor protein comprising the amino acid sequence shown in Fig. 2. In another embodiment, the function of the protein encoded by the nucleic acid analog differs from the function of the receptor protein comprising the amino acid sequence shown in Fig. 2. In another embodiment, the variation in the nucleic acid sequence occurs within the transmembrane (TM) region of the protein. In a further embodiment, the variation in the nucleic acid sequence occurs outside of the TM region.

This invention provides the above-described isolated nucleic acid, wherein the nucleic acid is DNA. In an embodiment, the DNA is cDNA. In another embodiment, the DNA is genomic DNA. In still another embodiment, the nucleic acid is RNA. Methods for production and manipulation of nucleic acid molecules are well known in the art.

This invention further provides nucleic acid which is degenerate with respect to the DNA encoding the polypeptides described herein. In an embodiment, the nucleic acid comprises a nucleotide sequence which is degenerate with respect to the nucleotides sequence shown in Figure 1 (SEQ ID NO: 2) or the nucleotide sequence contained in the plasmid pEXJ.HR-TL231, that is, a nucleotide sequence which is translated into the same

amino acid sequence.

This invention also encompasses DNAs and cDNAs which encode amino acid sequences which differ from those of the polypeptides of this invention, but which should not produce phenotypic changes. Alternately, this invention also encompasses DNAs, cDNAs, and RNAs which hybridize to the DNA, cDNA, and RNA of the subject invention. Hybridization methods are well known to those of skill in the art.

The nucleic acids of the subject invention also include nucleic acid molecules coding for polypeptide analogs, fragments or derivatives of antigenic polypeptides which differ from naturally-occurring forms in terms of the identity or location of one or more amino acid residues (deletion analogs containing less than all of the residues specified for the protein, substitution analogs wherein one or more residues specified are replaced by other residues and addition analogs wherein one or more amino acid residues is added to a terminal or medial portion of the polypeptides) and which share some or all properties of naturally-occurring forms. These molecules include: the incorporation of codons "preferred" for expression by selected non-mammalian hosts; the provision of sites for cleavage by restriction endonuclease enzymes; and the provision of additional initial, terminal or intermediate DNA sequences that facilitate construction of readily expressed vectors. The creation of polypeptide analogs is well known to those of skill in the art (R.F. Spurney et al. (1997); Fong, T.M. et al. (1995); Underwood, D.J. et al. (1994); Graziano, M.P. et al. (1996); Guan X.M. et al. (1995)).

The modified polypeptides of this invention may be

- transfected into cells either transiently or stably using methods well-known in the art, examples of which are disclosed herein. This invention also provides for binding assays using the modified polypeptides, in which the polypeptide is expressed either transiently or in stable cell lines. This invention further provides a compound identified using a modified polypeptide in a binding assay such as the binding assays described herein.
- The nucleic acids described and claimed herein are useful for the information which they provide concerning the amino acid sequence of the polypeptide and as products for the large scale synthesis of the polypeptides by a variety of recombinant techniques. The nucleic acid molecule is useful for generating new cloning and expression vectors, transformed and transfected prokaryotic and eukaryotic host cells, and new and useful methods for cultured growth of such host cells capable of expression of the polypeptide and related products.
- This invention provides an isolated nucleic acid encoding a human MCH1 receptor or a mutant of such human MCH1 receptor which is activated by MCH or an analog or homolog thereof. In one embodiment, the nucleic acid is DNA. In another embodiment, the DNA is cDNA. In another embodiment, the DNA is genomic DNA. In another embodiment, the nucleic acid is RNA.
- This invention also provides methods of using an isolated nucleic acid encoding species homologs of the MCH1 receptor encoded by the nucleic acid sequence shown in Fig. 1 (SEQ ID NO: 1) or encoded by the plasmid pEXJ.HR-TL231. In one embodiment, the nucleic acid encodes a mammalian MCH1 receptor homolog which has substantially the same amino acid sequence as does the MCH1 receptor

encoded by the plasmid pEXJ.HR-TL231. In another embodiment, the nucleic acid encodes a mammalian MCH1 receptor homolog which has above 65% amino acid identity to the MCH1 receptor encoded by the plasmid pEXJ.HR-TL231; preferably above 75% amino acid identity to the MCH1 receptor encoded by the plasmid pEXJ.HR-TL231; more preferably above 85% amino acid identity to the MCH1 receptor encoded by the plasmid pEXJ.HR-TL231; most preferably above 95% amino acid identity to the MCH1 receptor encoded by the plasmid pEXJ.HR-TL231. In another embodiment, the mammalian MCH1 receptor homolog has above 70% nucleic acid identity to the MCH1 receptor gene contained in plasmid pEXJ.HR-TL231; preferably above 80% nucleic acid identity to the MCH1 receptor gene contained in the plasmid pEXJ.HR-TL231; more preferably above 90% nucleic acid identity to the MCH1 receptor gene contained in the plasmid pEXJ.HR-TL231. Examples of methods for isolating and purifying species homologs are described elsewhere (e.g., U.S. Patent No. 5,602,024, WO94/14957, WO97/26853, WO98/15570).

In a separate embodiment of the present invention, the nucleic acid encodes a MCH1 receptor which has an amino acid sequence identical to that encoded by the plasmid pEXJ.HR-TL231. In a further embodiment, the MCH1 receptor comprises a sequence substantially the same as the amino acid sequence shown in Figure 2 (SEQ ID NO: 2). In another embodiment, the MCH1 receptor comprises an amino acid sequence as shown in Figure 2 (SEQ ID NO: 2).

In one embodiment, the mutant human MCH1 receptor comprises an amino acid sequence as shown in Figure 13 (SEQ ID NO: 26). In another embodiment, the mutant human MCH1 receptor comprises an amino acid sequence as shown in Figure 14 (SEQ ID NO: 27). In still another embodiment,

the mutant human MCH1 receptor comprises an amino acid sequence as shown in Figure 15 (SEQ ID NO: 28).

5 In separate embodiments, the human MCH1 receptor is encoded by the nucleic acid sequence shown in Figure 1 beginning with any of the three indicated start (ATG) codons.

10 This invention provides an isolated nucleic acid encoding a modified human MCH1 receptor, which differs from a human MCH1 receptor by having an amino acid(s) deletion, replacement, or addition in the third intracellular domain.

15 This invention provides a nucleic acid encoding a human MCH1 receptor, wherein the nucleic acid (a) hybridizes to a nucleic acid having the defined sequence shown in Figure 1 (SEQ ID NO: 1) under low stringency conditions or a sequence complementary thereto and (b) is further characterized by its ability to cause a change in the pH of a culture of CHO cells when a MCH1 ligand is added to the culture and the CHO cells contain the nucleic acid which hybridized to the nucleic acid having the defined sequence or its complement. Hybridization at low stringency is performed at 40°C in a hybridization buffer containing 25% formamide, 5X SCC, 7mM Tris, 1X Denhardt's, 25μl/ml salmon sperm DNA. Wash at 40°C in 0.1X SCC, 0.1% SDS. Changes in pH are measured through microphysiometric measurement of receptor mediated extracellular acidification rates. Because cellular metabolism is intricately involved in a broad range of cellular events (including receptor activation of multiple messenger pathways), the use of microphysiometric measurements of cell metabolism can in principle provide a generic assay of cellular activity arising from the activation of any

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receptor regardless of the specifics of the receptor's signaling pathway. General guidelines for transient receptor expression, cell preparation and microphysiometric recording are described elsewhere (Salon, J.A. and Owicki, J.A., 1996). Receptors and/or control vectors are transiently expressed in CHO-K1 cells, by liposome mediated transfection according to the manufacturers recommendations (LipofectAMINE, GibcoBRL, Gaithersburg, MD), and maintained in Ham's F-12 complete (10% serum). A total of 10 μ g of DNA is used to transfect each 75cm² flask which had been split 24 hours prior to the transfection and judged to be 70-80% confluent at the time of transfection. 24 hours post transfection, the cells are harvested and 3 x 10⁵ cells seeded into microphysiometer capsules. Cells are allowed to attach to the capsule membrane for an additional 24 hours; during the last 16 hours, the cells are switched to serum-free F-12 complete to minimize ill-defined metabolic stimulation caused by assorted serum factors. On the day of the experiment the cell capsules are transferred to the microphysiometer and allowed to equilibrate in recording media (low buffer RPMI 1640, no bicarbonate, no serum (Molecular Devices Corporation, Sunnyvale, CA) containing 0.1% fatty acid free BSA), during which a baseline measurement of basal metabolic activity is established. A standard recording protocol specifies a 100 μ l/min flow rate, with a 2 min total pump cycle which includes a 30 sec flow interruption during which the acidification rate measurement is taken. Ligand challenges involve a 1 min 20 sec exposure to the sample just prior to the first post challenge rate measurement being taken, followed by two additional pump cycles for a total of 5 min 20 sec sample exposure. Typically, drugs in a primary screen are presented to the cells at 10 μ M final concentration. Ligand samples are then washed out and the acidification

rates reported are expressed as a percentage increase of the peak response over the baseline rate observed just prior to challenge. An examples of a MCH ligand includes, but is not limited to, the endogenous MCH peptide.

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This invention provides a purified human MCH1 receptor protein.

10 This invention provides a vector comprising nucleic acid encoding a human MCH1 receptor. In an embodiment, the vector is adapted for expression in a cell which comprises the regulatory elements necessary for expression of the nucleic acid in the cell operatively linked to the nucleic acid encoding the human MCH1 receptor as to permit expression thereof. In separate embodiments, the cell is a bacterial cell, an amphibian cell, a yeast cell, an insect cell or a mammalian cell. In another embodiment, the vector is a baculovirus. In one embodiment, the vector is a plasmid.

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20 This invention provides a plasmid designated pEXJ.HR-TL231 (ATCC Accession No. 203197). This plasmid comprises the regulatory elements necessary for expression of DNA in a mammalian cell operatively linked to DNA encoding the human MCH1 receptor so as to permit expression thereof.

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30 This plasmid (pEXJ.HR-TL231) was deposited on September 17, 1998, with the American Type Culture Collection (ATCC), 12301 Parklawn Drive, Rockville, Maryland 20852, U.S.A. under the provisions of the Budapest Treaty for the International Recognition of the Deposit of Microorganisms for the Purposes of Patent Procedure and was accorded ATCC Accession No. 203197.

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This invention further provides for any vector or plasmid

which comprises modified untranslated sequences, which are beneficial for expression in desired host cells or for use in binding or functional assays. For example, a vector or plasmid with untranslated sequences of varying lengths may express differing amounts of the polypeptide depending upon the host cell used. In an embodiment, the vector or plasmid comprises the coding sequence of the polypeptide and the regulatory elements necessary for expression in the host cell.

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This invention provides a cell comprising a vector comprising a nucleic acid encoding the human MCH1 receptor. In an embodiment, the cell is a non-mammalian cell. In a further embodiment, the non-mammalian cell is a Xenopus oocyte cell or a Xenopus melanophore cell. In another embodiment, the cell is a mammalian cell. In a further embodiment, the mammalian cell is a COS-7 cell, a 293 human embryonic kidney cell, a NIH-3T3 cell, a LM(tk-) cell, a mouse Y1 cell, or a CHO cell.

This invention provides an insect cell comprising a vector adapted for expression in an insect cell which comprises a nucleic acid encoding a human MCH1 receptor. In another embodiment, the insect cell is an Sf9 cell, an Sf21 cell or a Trichoplusia ni 5B1-4 (HighFive) cell.

This invention provides a membrane preparation isolated from any one of the cells described above.

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This invention provides a nucleic acid probe comprising at least 15 nucleotides, which probe specifically hybridizes with a nucleic acid encoding a human MCH1 receptor, wherein the probe has a unique sequence corresponding to a sequence present within one of the two strands of the nucleic acid encoding a human MCH1 receptor present in

plasmid pEXJ.HR-TL231. This invention also provides a nucleic acid probe comprising at least 15 nucleotides, which probe specifically hybridizes with a nucleic acid encoding a human MCH1 receptor, wherein the probe has a unique sequence corresponding to a sequence present within
5 (a) the nucleic acid sequence shown in Figure 1 (SEQ ID NO: 1) or (b) the reverse complement thereto. In one embodiment, the nucleic acid is DNA. In another embodiment, the nucleic acid is RNA.

10

As used herein, the phrase "specifically hybridizing" means the ability of a nucleic acid molecule to recognize a nucleic acid sequence complementary to its own and to form double-helical segments through hydrogen bonding between complementary base pairs.
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Nucleic acid probe technology is well known to those skilled in the art who will readily appreciate that such probes may vary greatly in length and may be labeled with a detectable label, such as a radioisotope or fluorescent dye, to facilitate detection of the probe. DNA probe molecules may be produced by insertion of a DNA molecule which encodes the polypeptides of this invention into suitable vectors, such as plasmids or bacteriophages,
25 followed by transforming into suitable bacterial host cells, replication in the transformed bacterial host cells and harvesting of the DNA probes, using methods well known in the art. Alternatively, probes may be generated chemically from DNA synthesizers.

30

RNA probes may be generated by inserting the DNA molecule which encodes the polypeptides of this invention downstream of a bacteriophage promoter such as T3, T7, or SP6. Large amounts of RNA probe may be produced by incubating the labeled nucleotides with the linearized
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fragment where it contains an upstream promoter in the presence of the appropriate RNA polymerase.

5 This invention provides an antisense oligonucleotide having a sequence capable of specifically hybridizing to RNA encoding a human MCH1 receptor, so as to prevent translation of the RNA. This invention also provides an antisense oligonucleotide having a sequence capable of specifically hybridizing to genomic DNA encoding a human
10 MCH1 receptor. In one embodiment, the oligonucleotide comprises chemically modified nucleotides or nucleotide analogues.

15 This invention provides an antibody capable of binding to a human MCH1 receptor encoded by a nucleic acid encoding a human MCH1 receptor. This invention also provides an agent capable of competitively inhibiting the binding of the antibody to a human MCH1 receptor. In one embodiment, the antibody is a monoclonal antibody or antisera.

20 This invention provides a pharmaceutical composition comprising (a) an amount of the oligonucleotide capable of passing through a cell membrane and effective to reduce expression of a human MCH1 receptor and (b) a pharmaceutically acceptable carrier capable of passing through the cell membrane. In an embodiment, the oligonucleotide is coupled to a substance which inactivates mRNA. In a further embodiment, the substance which inactivates mRNA is a ribozyme. In another embodiment, the pharmaceutically acceptable carrier comprises a structure which binds to a human MCH1 receptor on a cell capable of being taken up by the cells after binding to the structure. In a further embodiment, the pharmaceutically acceptable carrier is capable of binding to a human MCH1 receptor which is specific for a selected
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cell type.

This invention provides a pharmaceutical composition which comprises an amount of an antibody effective to block binding of a ligand to a human MCH1 receptor and a pharmaceutically acceptable carrier.

As used herein, the phrase "pharmaceutically acceptable carrier" means any of the standard pharmaceutically acceptable carriers and is any pharmaceutical carrier known to those of ordinary skill in the art as useful in formulating pharmaceutical compositions. Examples include, but are not limited to, phosphate buffered saline, physiological saline, water, and emulsions, such as oil/water emulsions.

On December 24, 1997 the Food and Drug Administration of the United States Department of Health and Human Services published a guidance entitled "Q3C Impurities: Residual Solvent". The guidance recommends acceptable amounts of residual solvents in pharmaceuticals for the safety of the patient, and recommends the use of less toxic solvents in the manufacture of drug substances and dosage forms. Table 1 of the guidance lists "Class 1 Solvents". The guidance then states that the use of Class 1 Solvents should be avoided in the production of drug substances, excipients, or drug products unless their use can be strongly justified in a risk-benefit assessment. The guidance further states that Class 2 Solvents should be limited in order to protect patients from potentially adverse effects. The guidance characterized the following solvents as Class 1 Solvents: benzene, carbon tetrachloride, 1,2-dichloroethane, 1,1-dichloroethene, and 1,1,1-trichloroethane. The guidance characterized the following solvents as Class 2 Solvents: acetonitrile,

chlorobenzene, chloroform, cyclohexane, 1,2-dichloroethene, dichloromethane, 1,2-dimethoxyethane, N,N-dimethylacetamide, N,N-dimethylformamide, 1,4-dioxane, 2-ethoxyethanol, ethyleneglycol, formamide, hexane, 5 methanol, 2-methoxyethanol, methylbutyl ketone, methylcyclohexane, N-methylpyrrolidone, nitromethane, pyridine, sulfolane, tetralin, toluene, 1,1,2-trichloroethene and xylene. As used in this invention the term "pharmaceutically acceptable carrier" shall not 10 include Class 1 or Class 2 Solvents.

In an embodiment of the present invention, the pharmaceutical carrier may be a liquid and the pharmaceutical composition would be in the form of a 15 solution. In another embodiment, the pharmaceutically acceptable carrier is a solid and the composition is in the form of a powder or tablet. In a further embodiment, the pharmaceutical carrier is a gel and the composition is in the form of a suppository or cream. In a further 20 embodiment the compound may be formulated as a part of a pharmaceutically acceptable transdermal patch. In yet a further embodiment, the compound may be delivered to the subject by means of a spray or inhalant.

25 A solid carrier can include one or more substances which may also act as endogenous carriers (e.g. nutrient or micronutrient carriers), flavoring agents, lubricants, solubilizers, suspending agents, fillers, glidants, compression aids, binders or tablet-disintegrating agents; 30 it can also be an encapsulating material. In powders, the carrier is a finely divided solid which is in admixture with the finely divided active ingredient. In tablets, the active ingredient is mixed with a carrier having the necessary compression properties in suitable proportions 35 and compacted in the shape and size desired. The powders

and tablets preferably contain up to 99% of the active ingredient. Suitable solid carriers include, for example, calcium phosphate, magnesium stearate, talc, sugars, lactose, dextrin, starch, gelatin, cellulose, polyvinylpyrrolidone, low melting waxes and ion exchange resins.

Liquid carriers are used in preparing solutions, suspensions, emulsions, syrups, elixirs and pressurized compositions. The active ingredient can be dissolved or suspended in a pharmaceutically acceptable liquid carrier such as water, an organic solvent, a mixture of both or pharmaceutically acceptable oils or fats. The liquid carrier can contain other suitable pharmaceutical additives such as solubilizers, emulsifiers, buffers, preservatives, sweeteners, flavoring agents, suspending agents, thickening agents, colors, viscosity regulators, stabilizers or osmoregulators. Suitable examples of liquid carriers for oral and parenteral administration include water (partially containing additives as above, e.g. cellulose derivatives, preferably sodium carboxymethyl cellulose solution), alcohols (including monohydric alcohols and polyhydric alcohols, e.g. glycols) and their derivatives, and oils (e.g. fractionated coconut oil and arachis oil). For parenteral administration, the carrier can also be an oily ester such as ethyl oleate or isopropyl myristate. Sterile liquid carriers are useful in sterile liquid form compositions for parenteral administration. The liquid carrier for pressurized compositions can be halogenated hydrocarbon or other pharmaceutically acceptable propellant.

Liquid pharmaceutical compositions which are sterile solutions or suspensions can be utilized by for example, intramuscular, intrathecal, epidural, intraperitoneal or

- subcutaneous injection. Sterile solutions can also be administered intravenously. The compounds may be prepared as a sterile solid composition which may be dissolved or suspended at the time of administration using sterile water, saline, or other appropriate sterile injectable medium. Carriers are intended to include necessary and inert binders, suspending agents, lubricants, flavorants, sweeteners, preservatives, dyes, and coatings.
- The MCH1 antagonist can be administered orally in the form of a sterile solution or suspension containing other solutes or suspending agents (for example, enough saline or glucose to make the solution isotonic), bile salts, acacia, gelatin, sorbitan monoleate, polysorbate 80 (oleate esters of sorbitol and its anhydrides copolymerized with ethylene oxide) and the like.
- The MCH1 antagonist can also be administered orally either in liquid or solid composition form. Compositions suitable for oral administration include solid forms, such as pills, capsules, granules, tablets, and powders, and liquid forms, such as solutions, syrups, elixirs, and suspensions. Forms useful for parenteral administration include sterile solutions, emulsions, and suspensions.
- Optimal dosages to be administered may be determined by those skilled in the art, and will vary with the particular compound in use, the strength of the preparation, the mode of administration, and the advancement of the disease condition. Additional factors depending on the particular subject being treated will result in a need to adjust dosages, including subject age, weight, gender, diet, and time of administration.

This invention provides a transgenic, nonhuman mammal expressing DNA encoding a human MCH1 receptor. This invention also provides a transgenic, nonhuman mammal comprising a homologous recombination knockout of the native human MCH1 receptor. This invention further provides a transgenic, nonhuman mammal whose genome comprises antisense DNA complementary to the DNA encoding a human MCH1 receptor so placed within the genome as to be transcribed into antisense mRNA which is complementary to mRNA encoding the human MCH1 receptor and which hybridizes to mRNA encoding the human MCH1 receptor, thereby reducing its translation. In an embodiment, the DNA encoding the human MCH1 receptor additionally comprises an inducible promoter. In another embodiment, the DNA encoding the human MCH1 receptor additionally comprises tissue specific regulatory elements. In a further embodiment, the transgenic, nonhuman mammal is a mouse.

Animal model systems which elucidate the physiological and behavioral roles of the polypeptides of this invention are produced by creating transgenic animals in which the activity of the polypeptide is either increased or decreased, or the amino acid sequence of the expressed polypeptide is altered, by a variety of techniques. Examples of these techniques include, but are not limited to: 1) Insertion of normal or mutant versions of DNA encoding the polypeptide, by microinjection, electroporation, retroviral transfection or other means well known to those in the art, into appropriate fertilized embryos in order to produce a transgenic animal or 2) Homologous recombination of mutant or normal, human or animal versions of these genes with the native gene locus in transgenic animals to alter the regulation of expression or the structure of these polypeptide sequences. The technique of homologous recombination is

well known in the art. It replaces the native gene with the inserted gene and so is useful for producing an animal that cannot express native polypeptides but does express, for example, an inserted mutant polypeptide, which has replaced the native polypeptide in the animal's genome by recombination, resulting in underexpression of the transporter. Microinjection adds genes to the genome, but does not remove them, and so is useful for producing an animal which expresses its own and added polypeptides, resulting in overexpression of the polypeptides.

One means available for producing a transgenic animal, with a mouse as an example, is as follows: Female mice are mated, and the resulting fertilized eggs are dissected out of their oviducts. The eggs are stored in an appropriate medium such as M2 medium. DNA or cDNA encoding a polypeptide of this invention is purified from a vector by methods well known in the art. Inducible promoters may be fused with the coding region of the DNA to provide an experimental means to regulate expression of the trans-gene. Alternatively, or in addition, tissue specific regulatory elements may be fused with the coding region to permit tissue-specific expression of the trans-gene. The DNA, in an appropriately buffered solution, is put into a microinjection needle (which may be made from capillary tubing using a pipette puller) and the egg to be injected is put in a depression slide. The needle is inserted into the pronucleus of the egg, and the DNA solution is injected. The injected egg is then transferred into the oviduct of a pseudopregnant mouse (a mouse stimulated by the appropriate hormones to maintain pregnancy but which is not actually pregnant), where it proceeds to the uterus, implants, and develops to term. As noted above, microinjection is not the only method for inserting DNA

into the egg cell, and is used here only for exemplary purposes.

This invention provides a process for identifying a
5 chemical compound which specifically binds to a mammalian
MCH1 receptor which comprises contacting cells comprising
DNA encoding, and expressing on their cell surface, the
mammalian MCH1 receptor, with the compound under
conditions suitable for binding, and detecting specific
10 binding of the chemical compound to the mammalian MCH1
receptor, wherein the cells do not normally express the
mammalian MCH1 receptor and the DNA encoding the mammalian
MCH1 receptor (a) hybridizes to a nucleic acid having the
defined sequence shown in Figure 1 (SEQ ID NO: 1) under
15 low stringency conditions or a sequence complementary
thereto and (b) is further characterized by its ability to
cause a change in the pH of a culture of CHO cells when a
MCH1 ligand is added to the culture and the CHO cells
contain the nucleic acid which hybridized to the nucleic
20 acid having the defined sequence or its complement. This
invention also provides a process for identifying a
chemical compound which specifically binds to a mammalian
MCH1 receptor which comprises contacting a membrane
preparation from cells comprising DNA encoding, and
25 expressing on their cell surface, the mammalian MCH1 receptor,
with the compound under conditions suitable for
binding, and detecting specific binding of the chemical
compound to the mammalian MCH1 receptor, wherein the cells
do not normally express the mammalian MCH1 receptor and
30 the DNA encoding the mammalian MCH1 receptor (a)
hybridizes to a nucleic acid having the defined sequence
shown in Figure 1 (SEQ ID NO: 1) under low stringency
conditions or a sequence complementary thereto and (b) is
further characterized by its ability to cause a change in
35 the pH of a culture of CHO cells when a MCH1 ligand is

added to the culture and the CHO cells contain the nucleic acid which hybridized to the nucleic acid having the defined sequence or its complement. In one embodiment, the MCH1 receptor is a human MCH1 receptor. In another embodiment, the MCH1 receptor is a rat MCH1 receptor. In another embodiment, the mammalian MCH1 receptor comprises substantially the same amino acid sequence as the sequence of the human MCH1 receptor encoded by plasmid pEXJ.HR-TL231. In a further embodiment, the mammalian MCH1 receptor comprises substantially the same amino acid sequence as that shown in Figure 2 (SEQ ID NO: 2). In another embodiment, the mammalian MCH1 receptor comprises the amino acid sequence shown in Figure 2 (SEQ ID NO: 2). In a different embodiment, the mammalian MCH1 receptor comprises the amino acid sequence shown in Figure 13 (SEQ ID NO: 26). In another embodiment, the mammalian MCH1 receptor comprises the amino acid sequence shown in Figure 14 (SEQ ID NO: 27). In still another embodiment, the mammalian MCH1 receptor comprises the amino acid sequence shown in Figure 15 (SEQ ID NO: 28). In one embodiment, the compound is not previously known to bind to a mammalian MCH1 receptor. This invention further provides a compound identified by the above-described processes.

In one embodiment of the above-described processes, the cell is an insect cell. In another embodiment, the cell is a mammalian cell. In a further embodiment, the cell is nonneuronal in origin. In a further embodiment, the nonneuronal cell is a COS-7 cell, 293 human embryonic kidney cell, a CHO cell, a NIH-3T3 cell, a mouse Y1 cell, or a LM(tk-) cell.

This invention provides a process involving competitive binding for identifying a chemical compound which specifically binds to a mammalian MCH1 receptor which

comprises contacting cells expressing on their cell surface the mammalian MCH1 receptor, with both the chemical compound and a second chemical compound known to bind to the receptor, and separately with only the second chemical compound, under conditions suitable for binding of both compounds, and detecting specific binding of the chemical compound to the mammalian MCH1 receptor, a decrease in the binding of the second chemical compound to the mammalian MCH1 receptor in the presence of the chemical compound indicating that the chemical compound binds to the mammalian MCH1 receptor, wherein the cells do not normally express the mammalian MCH1 receptor and the DNA encoding the mammalian MCH1 receptor (a) hybridizes to a nucleic acid having the defined sequence shown in Figure 1 (SEQ ID NO: 1) under low stringency conditions or a sequence complementary thereto and (b) is further characterized by its ability to cause a change in the pH of a culture of CHO cells when a MCH1 ligand is added to the culture and the CHO cells contain the nucleic acid which hybridized to the nucleic acid having the defined sequence or its complement.

This invention also provides a process involving competitive binding for identifying a chemical compound which specifically binds to a mammalian MCH1 receptor which comprises contacting a membrane preparation from cells expressing on their cell surface the mammalian MCH1 receptor, with both the chemical compound and a second chemical compound known to bind to the receptor, and separately with only the second chemical compound, under conditions suitable for binding of both compounds, and detecting specific binding of the chemical compound to the mammalian MCH1 receptor, a decrease in the binding of the second chemical compound to the mammalian MCH1 receptor in the presence of the chemical compound indicating that the

chemical compound binds to the mammalian MCH1 receptor, wherein the cells do not normally express the mammalian MCH1 receptor and the DNA encoding the mammalian MCH1 receptor (a) hybridizes to a nucleic acid having the defined sequence shown in Figure 1 (SEQ ID NO: 1) under low stringency conditions or a sequence complementary thereto and (b) is further characterized by its ability to cause a change in the pH of a culture of CHO cells when a MCH1 ligand is added to the culture and the CHO cells contain the nucleic acid which hybridized to the nucleic acid having the defined sequence or its complement.

In one embodiment, the mammalian MCH1 receptor is a human MCH1 receptor or a mutant of such human MCH1 receptor which is activated by MCH or an analog or homolog thereof. In another embodiment, the mammalian MCH1 receptor is a rat MCH1 receptor. In another embodiment, the mammalian MCH1 receptor comprises substantially the same amino acid sequence as the human MCH1 receptor encoded by plasmid pEXJ.HR-TL231. In a further embodiment, the mammalian MCH1 receptor comprises substantially the same amino acid sequence as that shown in Figure 2 (SEQ ID NO: 2). In another embodiment, the mammalian MCH1 receptor comprises the amino acid sequence shown in Figure 2 (SEQ ID NO: 2).

In one embodiment, the cell is an insect cell. In another embodiment, the cell is a mammalian cell. In a further embodiment, the cell is nonneuronal in origin. In another embodiment, the nonneuronal cell is a COS-7 cell, 293 human embryonic kidney cell, a CHO cell, a NIH-3T3 cell, a mouse Y1 cell, or a LM(tk-) cell. In one embodiment, the compound is not previously known to bind to a mammalian MCH1 receptor.

This invention provides a compound identified by the above-described processes.

5 This invention provides a method of screening a plurality
of chemical compounds not known to bind to a mammalian
MCH1 receptor to identify a compound which specifically
binds to the mammalian MCH1 receptor, which comprises (a)
contacting cells transfected with and expressing DNA
encoding the mammalian MCH1 receptor with the plurality of
10 compounds not known to bind specifically to the mammalian
MCH1 receptor, under conditions permitting binding of
compounds known to bind the mammalian MCH1 receptor; (b)
determining whether the binding of a compound known to
bind to the mammalian MCH1 receptor is reduced in the
15 presence of the compounds within the plurality of
compounds, relative to the binding of the compound in the
absence of the plurality of compounds; and if so (c)
separately determining the binding to the mammalian MCH1
receptor of compounds included in the plurality of
20 compounds, so as to thereby identify the compound which
specifically binds to the mammalian MCH1 receptor.

25 This invention provides a method of screening a plurality
of chemical compounds not known to bind to a mammalian
MCH1 receptor to identify a compound which specifically
binds to the mammalian MCH1 receptor, which comprises (a)
contacting a membrane preparation from cells transfected
with and expressing the mammalian MCH1 receptor with the
plurality of compounds not known to bind specifically to
30 the mammalian MCH1 receptor, under conditions permitting
binding of compounds known to bind the mammalian MCH1
receptor; (b) determining whether the binding of a
compound known to bind to the mammalian MCH1 receptor is
reduced in the presence of the compounds within the
35 plurality of compounds, relative to the binding of the

compound in the absence of the plurality of compounds; and if so (c) separately determining the binding to the mammalian MCH1 receptor of compounds included in the plurality of compounds, so as to thereby identify the compound which specifically binds to the mammalian MCH1 receptor.

In one embodiment of the above-described methods, the mammalian MCH1 receptor is a human MCH1 receptor or a mutant of such human MCH1 receptor which is activated by MCH or an analog or homolog thereof. In another embodiment, the mammalian MCH1 receptor is a rat MCH1 receptor. In another embodiment, the cell is a mammalian cell. In a further embodiment, the mammalian cell is non-neuronal in origin. In another embodiment, the non-neuronal cell is a COS-7 cell, a 293 human embryonic kidney cell, a LM(tk-) cell, a CHO cell, a mouse Y1 cell, or an NIH-3T3 cell.

This invention also provides a method of detecting expression of a mammalian MCH1 receptor by detecting the presence of mRNA coding for the mammalian MCH1 receptor which comprises obtaining total mRNA from the cell and contacting the mRNA so obtained from a nucleic acid probe under hybridizing conditions, detecting the presence of mRNA hybridizing to the probe, and thereby detecting the expression of the mammalian MCH1 receptor by the cell.

This invention further provides a method of detecting the presence of a mammalian MCH1 receptor on the surface of a cell which comprises contacting the cell with an antibody under conditions permitting binding of the antibody to the receptor, detecting the presence of the antibody bound to the cell, and thereby detecting the presence of the mammalian MCH1 receptor on the surface of the cell.

This invention provides a method of determining the physiological effects of varying levels of activity of human MCH1 receptors which comprises producing a transgenic, nonhuman mammal whose levels of human MCH1 receptor activity are varied by use of an inducible promoter which regulates human MCH1 receptor expression.

This invention also provides a method of determining the physiological effects of varying levels of activity of human MCH1 receptors which comprises producing a panel of transgenic, nonhuman mammals each expressing a different amount of human MCH1 receptor.

This invention provides a method for identifying an antagonist capable of alleviating an abnormality wherein the abnormality is alleviated by decreasing the activity of a human MCH1 receptor comprising administering a compound to a transgenic, nonhuman mammal, and determining whether the compound alleviates the physical and behavioral abnormalities displayed by the transgenic, nonhuman mammal as a result of overactivity of a human MCH1 receptor, the alleviation of the abnormality identifying the compound as an antagonist. This invention also provides an antagonist identified by the above-described method. This invention further provides a pharmaceutical composition comprising an antagonist identified by the above-described method and a pharmaceutically acceptable carrier. This invention provides a method of treating an abnormality in a subject wherein the abnormality is alleviated by decreasing the activity of a human MCH1 receptor which comprises administering to the subject an effective amount of this pharmaceutical composition, thereby treating the abnormality.

This invention provides a method for identifying an agonist capable of alleviating an abnormality in a subject wherein the abnormality is alleviated by increasing the activity of a human MCH1 receptor comprising administering a compound to transgenic, nonhuman mammal, and determining whether the compound alleviates the physical and behavioral abnormalities displayed by the transgenic, nonhuman mammal, the alleviation of the abnormality identifying the compound as an agonist. This invention also provides an agonist identified by the above-described method. This invention further provides a pharmaceutical composition comprising an agonist identified by the above-described method and a pharmaceutically acceptable carrier. This invention further provides a method of treating an abnormality in a subject wherein the abnormality is alleviated by increasing the activity of a human MCH1 receptor which comprises administering to the subject an effective amount of this pharmaceutical composition, thereby treating the abnormality.

This invention provides a method for diagnosing a predisposition to a disorder associated with the activity of a specific mammalian allele which comprises: (a) obtaining DNA of subjects suffering from the disorder; (b) performing a restriction digest of the DNA with a panel of restriction enzymes; (c) electrophoretically separating the resulting DNA fragments on a sizing gel; (d) contacting the resulting gel with a nucleic acid probe capable of specifically hybridizing with a unique sequence included within the sequence of a nucleic acid molecule encoding a human MCH1 receptor and labeled with a detectable marker; (e) detecting labeled bands which have hybridized to the DNA encoding a human MCH1 receptor labeled with a detectable marker to create a unique band pattern specific to the DNA of subjects suffering from the

disorder; (f) preparing DNA obtained for diagnosis by steps (a)-(e); and (g) comparing the unique band pattern specific to the DNA of subjects suffering from the disorder from step (e) and the DNA obtained for diagnosis from step (f) to determine whether the patterns are the same or different and to diagnose thereby predisposition to the disorder if the patterns are the same. In one embodiment, a disorder associated with the activity of a specific mammalian allele is diagnosed.

This invention provides a method of preparing the purified human MCH1 receptor which comprises: (a) inducing cells to express the human MCH1 receptor; (b) recovering the human MCH1 receptor from the induced cells; and (c) purifying the human MCH1 receptor so recovered.

This invention provides a method of preparing the purified human MCH1 receptor which comprises: (a) inserting nucleic acid encoding the human MCH1 receptor in a suitable vector; (b) introducing the resulting vector in a suitable host cell; (c) placing the resulting cell in suitable condition permitting the production of the isolated human MCH1 receptor; (d) recovering the human MCH1 receptor produced by the resulting cell; and (e) purifying the human MCH1 receptor so recovered.

This invention provides a process for determining whether a chemical compound is a mammalian MCH1 receptor agonist which comprises contacting cells transfected with and expressing DNA encoding the mammalian MCH1 receptor with the compound under conditions permitting the activation of the mammalian MCH1 receptor, and detecting an increase in mammalian MCH1 receptor activity, so as to thereby determine whether the compound is a mammalian MCH1 receptor agonist. This invention also provides a process

for determining whether a chemical compound is a mammalian MCH1 receptor antagonist which comprises contacting cells transfected with and expressing DNA encoding the mammalian MCH1 receptor with the compound in the presence of a known mammalian MCH1 receptor agonist, under conditions permitting the activation of the mammalian MCH1 receptor, and detecting a decrease in mammalian MCH1 receptor activity, so as to thereby determine whether the compound is a mammalian MCH1 receptor antagonist. In one embodiment, the mammalian MCH1 receptor is a human MCH1 receptor or a mutant of such human MCH1 receptor which is activated by MCH or an analog or homolog thereof.

This invention further provides a pharmaceutical composition which comprises an amount of a mammalian MCH1 receptor agonist determined by the above-described process effective to increase activity of a mammalian MCH1 receptor and a pharmaceutically acceptable carrier. In one embodiment, the mammalian MCH1 receptor agonist is not previously known.

This invention provides a pharmaceutical composition which comprises an amount of a mammalian MCH1 receptor antagonist determined by the above-described process effective to reduce activity of a mammalian MCH1 receptor and a pharmaceutically acceptable carrier. In one embodiment, the mammalian MCH1 receptor antagonist is not previously known.

This invention provides a process for determining whether a chemical compound specifically binds to and activates a mammalian MCH1 receptor, which comprises contacting cells producing a second messenger response and expressing on their cell surface the mammalian MCH1 receptor, wherein such cells do not normally express the mammalian MCH1

receptor, with the chemical compound under conditions suitable for activation of the mammalian MCH1 receptor, and measuring the second messenger response in the presence and in the absence of the chemical compound, a change in the second messenger response in the presence of the chemical compound indicating that the compound activates the mammalian MCH1 receptor. In one embodiment, the second messenger response comprises chloride channel activation and the change in second messenger is an increase in the level of inward chloride current.

This invention also provides a process for determining whether a chemical compound specifically binds to and inhibits activation of a mammalian MCH1 receptor, which comprises separately contacting cells producing a second messenger response and expressing on their cell surface the mammalian MCH1 receptor, wherein such cells do not normally express the mammalian MCH1 receptor, with both the chemical compound and a second chemical compound known to activate the mammalian MCH1 receptor, and with only the second chemical compound, under conditions suitable for activation of the mammalian MCH1 receptor, and measuring the second messenger response in the presence of only the second chemical compound and in the presence of both the second chemical compound and the chemical compound, a smaller change in the second messenger response in the presence of both the chemical compound and the second chemical compound than in the presence of only the second chemical compound indicating that the chemical compound inhibits activation of the mammalian MCH1 receptor. In one embodiment, the second messenger response comprises chloride channel activation and the change in second messenger response is a smaller increase in the level of inward chloride current in the presence of both the chemical compound and the second chemical compound than in

the presence of only the second chemical compound. This invention also provides the above-described processes performed with membrane preparations from cells producing a second messenger response and transfected with and expressing the mammalian MCH1 receptor.

In one embodiment of the above-described processes, the mammalian MCH1 receptor is a human MCH1 receptor or a mutant of such human MCH1 receptor which is activated by MCH or an analog or homolog thereof. In another embodiment, the mammalian MCH1 receptor is a rat MCH1 receptor. In another embodiment, the mammalian MCH1 receptor comprises substantially the same amino acid sequence as encoded by the plasmid pEXJ.HR-TL231. In a further embodiment, the mammalian MCH1 receptor comprises substantially the same amino acid sequence as that shown in Figure 2 (SEQ ID NO: 2). In another embodiment, the mammalian MCH1 receptor comprises an amino acid sequence as shown in Figure 2 (SEQ ID NO: 2). In an embodiment, the cell is an insect cell. In a further embodiment, the cell is a mammalian cell. In a still further embodiment, the mammalian cell is nonneuronal in origin. In another embodiment, the nonneuronal cell is a COS-7 cell, CHO cell, 293 human embryonic kidney cell, NIH-3T3 cell or LM(tk-) cell. In an embodiment, the compound is not previously known to bind to a mammalian MCH1 receptor. This invention also provides a compound determined by the above-described processes.

This invention also provides a pharmaceutical composition which comprises an amount of a mammalian MCH1 receptor agonist determined by the above-described processes effective to increase activity of a mammalian MCH1 receptor and a pharmaceutically acceptable carrier. In

one embodiment, the mammalian MCH1 receptor agonist is not previously known.

5 This invention further provides a pharmaceutical composition which comprises an amount of a mammalian MCH1 receptor antagonist determined by the above-described processes effective to reduce activity of a mammalian MCH1 receptor and a pharmaceutically acceptable carrier. In one embodiment, the mammalian MCH1 receptor antagonist is
10 not previously known.

This invention provides a method of screening a plurality of chemical compounds not known to activate a mammalian MCH1 receptor to identify a compound which activates the mammalian MCH1 receptor which comprises: (a) contacting cells transfected with and expressing the mammalian MCH1 receptor with the plurality of compounds not known to activate the mammalian MCH1 receptor, under conditions permitting activation of the mammalian MCH1 receptor; (b) determining whether the activity of the mammalian MCH1 receptor is increased in the presence of the compounds; and if so (c) separately determining whether the activation of the mammalian MCH1 receptor is increased by each compound included in the plurality of compounds, so as to thereby identify the compound which activates the mammalian MCH1 receptor. In one embodiment, the mammalian MCH1 receptor is a human MCH1 receptor or a mutant of such human MCH1 receptor which is activated by MCH or an analog or homolog thereof. In another embodiment, the mammalian MCH1 receptor is a rat MCH1 receptor.
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35 This invention provides a method of screening a plurality of chemical compounds not known to inhibit the activation of a mammalian MCH1 receptor to identify a compound which

inhibits the activation of the mammalian MCH1 receptor, which comprises: (a) contacting cells transfected with and expressing the mammalian MCH1 receptor with the plurality of compounds in the presence of a known mammalian MCH1 receptor agonist, under conditions permitting activation of the mammalian MCH1 receptor; (b) determining whether the activation of the mammalian MCH1 receptor is reduced in the presence of the plurality of compounds, relative to the activation of the mammalian MCH1 receptor in the absence of the plurality of compounds; and if so (c) separately determining the inhibition of activation of the mammalian MCH1 receptor for each compound included in the plurality of compounds, so as to thereby identify the compound which inhibits the activation of the mammalian MCH1 receptor. In one embodiment, the mammalian MCH1 receptor is a human MCH1 receptor or a mutant of such human MCH1 receptor which is activated by MCH or an analog or homolog thereof. In another embodiment, the mammalian MCH1 receptor is a rat MCH1 receptor.

In one embodiment of the above-described methods, the cell is a mammalian cell. In another embodiment, the mammalian cell is non-neuronal in origin. In a further embodiment, the non-neuronal cell is a COS-7 cell, a 293 human embryonic kidney cell, a LM(tk-) cell or an NIH-3T3 cell.

This invention provides a pharmaceutical composition comprising a compound identified by the above-described methods effective to increase mammalian MCH1 receptor activity and a pharmaceutically acceptable carrier.

This invention also provides a pharmaceutical composition comprising a compound identified by the above-described methods effective to decrease mammalian MCH1 receptor activity and a pharmaceutically acceptable carrier.

This invention further provides a method of measuring receptor activation in an oocyte expression system such as a *Xenopus* oocyte expression system or melanophore. In an embodiment, receptor activation is determined by measurement of ion channel activity. In another embodiment, receptor activation is measured by aequorin luminescence.

Expression of genes in *Xenopus* oocytes is well known in the art (Coleman, A., 1984; Masu, Y., et al., 1994) and is performed using microinjection of native mRNA or in vitro synthesized mRNA into frog oocytes. The preparation of in vitro synthesized mRNA can be performed by various standard techniques (Sambrook, et al. 1989) including using T7 polymerase with the mCAP RNA mapping kit (Stratagene).

This invention provides a method of treating an abnormality in a subject wherein the abnormality is alleviated by increasing the activity of a mammalian MCH1 receptor which comprises administering to the subject an amount of a compound which is a mammalian MCH1 receptor agonist effective to treat the abnormality. In separate embodiments, the abnormality is a regulation of a steroid or pituitary hormone disorder, an epinephrine release disorder, a gastrointestinal disorder, a cardiovascular disorder, an electrolyte balance disorder, hypertension, diabetes, a respiratory disorder, asthma, a reproductive function disorder, an immune disorder, an endocrine disorder, a musculoskeletal disorder, a neuroendocrine disorder, a cognitive disorder, a memory disorder such as Alzheimer's disease, a sensory modulation and transmission disorder, a motor coordination disorder, a sensory integration disorder, a motor integration disorder, a dopaminergic function disorder such as Parkinson's

5 disease, a sensory transmission disorder, an olfaction disorder, a sympathetic innervation disorder, an affective disorder such as depression, a stress-related disorder, a fluid-balance disorder, a urinary disorder such as urinary incontinence, a seizure disorder, pain, psychotic behavior such as schizophrenia, morphine tolerance, opiate addiction or migraine.

10 This invention provides a method of treating an abnormality in a subject wherein the abnormality is alleviated by decreasing the activity of a mammalian MCH1 receptor which comprises administering to the subject an amount of a compound which is a mammalian MCH1 receptor antagonist effective to treat the abnormality. In separate embodiments, the abnormality is a regulation of a steroid or pituitary hormone disorder, an epinephrine release disorder, a gastrointestinal disorder, a cardiovascular disorder, an electrolyte balance disorder, hypertension, diabetes, a respiratory disorder, asthma, a reproductive function disorder, an immune disorder, an endocrine disorder, a musculoskeletal disorder, a neuroendocrine disorder, a cognitive disorder, a memory disorder such as Alzheimer's disease, a sensory modulation and transmission disorder, a motor coordination disorder, a sensory integration disorder, a motor integration disorder, a dopaminergic function disorder such as Parkinson's disease, a sensory transmission disorder, an olfaction disorder, a sympathetic innervation disorder, an affective disorder such as depression, a stress-related disorder, a fluid-balance disorder, a urinary disorder such as urinary incontinence, a seizure disorder, pain, psychotic behavior such as schizophrenia, morphine tolerance, opiate addiction or migraine.

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- This invention provides a process for making a composition of matter which specifically binds to a mammalian MCH1 receptor which comprises identifying a chemical compound using any of the processes described herein for identifying a compound which binds to and/or activates or inhibits activation of a mammalian MCH1 receptor and then synthesizing the chemical compound or a novel structural and functional analog or homolog thereof. In one embodiment, the mammalian MCH1 receptor is a human MCH1 receptor or a mutant of such human MCH1 receptor which is activated by MCH or an analog or homolog thereof. In another embodiment, the mammalian MCH1 receptor is a rat MCH1 receptor.
- This invention further provides a process for preparing a composition which comprises admixing a pharmaceutically acceptable carrier and a therapeutically effective amount of a chemical compound identified by any of the processes described herein for identifying a compound which binds to and/or activates or inhibits activation of a mammalian MCH1 receptor or a novel structural and functional analog or homolog thereof. In one embodiment, the mammalian MCH1 receptor is a human MCH1 receptor or a mutant of such human MCH1 receptor which is activated by MCH or an analog or homolog thereof. In another embodiment, the mammalian MCH1 receptor is a rat MCH1 receptor.
- This invention provides a process for determining whether a chemical compound is a human MCH1 receptor antagonist which comprises contacting cells transfected with and expressing DNA encoding the human MCH1 receptor with the compound in the presence of a known human MCH1 receptor agonist, under conditions permitting the activation of the human MCH1 receptor, and detecting a decrease in human MCH1 receptor activity, so as to thereby determine whether

the compound is a human MCH1 receptor antagonist, wherein
the DNA encoding the human MCH1 receptor comprises the
sequence shown in Figure 1 (Seq. ID No. 1) or contained in
plasmid pEXJ.HR-TL231 (ATCC Accession No. 203197), the
known human MCH1 receptor agonist is MCH or a homolog or
analog of MCH, and the cells do not express the MCH1
receptor prior to transfecting them.

This invention also provides a process for determining
whether a chemical compound specifically binds to and
inhibits activation of a human MCH1 receptor, which
comprises separately contacting cells expressing on their
cell surface the human MCH1 receptor and producing a
second messenger response upon activation of the human
MCH1 receptor, wherein such cells do not normally express
the human MCH1 receptor and the DNA encoding the human
MCH1 receptor comprises the sequence shown in Figure 1
(Seq. ID No. 1) or contained in plasmid pEXJ.HR-TL231
(ATCC Accession No. 203197), with both the chemical
compound and a second chemical compound known to activate
the human MCH1 receptor, and with only the second chemical
compound, under conditions suitable for activation of the
human MCH1 receptor, and measuring the second messenger
response in the presence of only the second chemical
compound and in the presence of both the second chemical
compound and the chemical compound, a smaller change in
the second messenger response in the presence of both the
chemical compound and the second chemical compound than in
the presence of only the second chemical compound
indicating that the chemical compound inhibits activation
of the human MCH1 receptor, wherein the second chemical
compound is MCH or a homolog or analog of MCH. In one
embodiment, the second messenger response comprises
chloride channel activation and the change in second
messenger response is a smaller increase in the level of

inward chloride current in the presence of both the chemical compound and the second chemical compound than in the presence of only the second chemical compound.

5 This invention further provides a method of screening a plurality of chemical compounds not known to inhibit the activation of a human MCH1 receptor to identify a compound which inhibits the activation of the human MCH1 receptor, which comprises:

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- (a) contacting cells transfected with and expressing the human MCH1 receptor, wherein such cells do not normally express the human MCH1 receptor and the DNA encoding the human MCH1 receptor comprises the sequence shown in Figure 1 (Seq. ID No. 1) or contained in plasmid pEXJ.HR-TL231 (ATCC Accession No. 203197), with the plurality of compounds in the presence of a known human MCH1 receptor agonist, under conditions permitting activation of the human MCH1 receptor, wherein the known MCH1 receptor agonist is MCH or a homolog or analog of MCH;
- (b) determining whether the activation of the human MCH1 receptor is reduced in the presence of the plurality of compounds, relative to the activation of the human MCH1 receptor in the absence of the plurality of compounds; and if so
- (c) separately determining the extent of inhibition of activation of the human MCH1 receptor for each compound included in the plurality of compounds, so as to thereby identify the compound which inhibits the activation of the human MCH1 receptor.

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In one embodiment of the above-described methods, the cell
is an insect cell. In another embodiment, the cell is a
mammalian cell. In still another embodiment, the cell is
a mammalian cell which is nonneuronal in origin. In
5 further embodiments, the cell is a COS-7 cell, a CHO cell,
a 293 human embryonic kidney cell, a NIH-3T3 cell, a mouse
Y1 cell, or a LM(tk-) cell.

This invention provides a process for making a composition
10 of matter which specifically binds to a human MCH1
receptor which comprises identifying a chemical compound
which specifically binds to the human MCH1 receptor and
then synthesizing the chemical compound or a structural
and functional analog or homolog thereof, wherein the
15 chemical compound is identified as binding to the human
MCH1 receptor by a process involving competitive binding
which comprises contacting cells expressing on their cell
surface the human MCH1 receptor, with both the chemical
compound and a second chemical compound known to bind to
20 the receptor, and separately with only the second chemical
compound, under conditions suitable for binding of both
compounds, and detecting the extent of specific binding of
the chemical compound to the human MCH1 receptor, a
decrease in the binding of the second chemical compound to
25 the human MCH1 receptor in the presence of the chemical
compound indicating that the chemical compound binds to
the human MCH1 receptor, wherein the cells do not normally
express the human MCH1 receptor, the human MCH1 receptor
is encoded by nucleic acid comprising the sequence shown
30 in Figure 1 (Seq. ID No. 1) or contained in plasmid
pEXJ.HR-TL231 (ATCC Accession No. 203197), and the second
chemical compound is MCH or a homolog or analog of MCH.

This invention further provides a process for making a
35 composition of matter which specifically binds to a human

MCH1 receptor which comprises identifying a chemical compound which specifically binds to the human MCH1 receptor and then synthesizing the chemical compound or a structural and functional analog or homolog thereof,
5 wherein the chemical compound is identified as binding to the human MCH1 receptor by a process involving competitive binding which comprises contacting a membrane preparation from cells expressing on their cell surface the human MCH1 receptor, with both the chemical compound and a second
10 chemical compound known to bind to the receptor, and separately with only the second chemical compound, under conditions suitable for binding of both compounds, and detecting the extent of specific binding of the chemical compound to the human MCH1 receptor, a decrease in the
15 binding of the second chemical compound to the human MCH1 receptor in the presence of the chemical compound indicating that the chemical compound binds to the human MCH1 receptor, wherein the cells do not normally express the human MCH1 receptor, the human MCH1 receptor is encoded by nucleic acid comprising the sequence shown in
20 Figure 1 (Seq. ID No. 1) or contained in plasmid pEXJ.HR-TL231 (ATCC Accession No. 203197), and the second chemical compound is MCH or a homolog or analog of MCH.

25 This invention also provides a process for making a composition of matter which is a human MCH1 receptor antagonist which comprises identifying a chemical compound which is a human MCH1 receptor antagonist and then synthesizing the chemical compound or a structural and functional analog or homolog thereof, wherein the chemical compound is identified as a human MCH1 receptor antagonist by a process which comprises contacting cells transfected with and expressing DNA encoding the human MCH1 receptor with the compound in the presence of a known human MCH1 receptor agonist, under conditions permitting the
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activation of the human MCH1 receptor, and detecting a decrease in human MCH1 receptor activity, so as to thereby determine whether the compound is a human MCH1 receptor antagonist, wherein the cells do not normally express the human MCH1 receptor, the human MCH1 receptor is encoded by nucleic acid comprising the sequence shown in Figure 1 (Seq. ID No. 1) or contained in plasmid pEXJ.HR-TL231 (ATCC Accession No. 203197), and the known human MCH1 receptor agonist is MCH or a homolog or analog of MCH.

This invention still further provides a process for making a composition of matter which specifically binds to and inhibits the activation of a human MCH1 receptor which comprises identifying a chemical compound which specifically binds to and inhibits the activation of the human MCH1 receptor and then synthesizing the chemical compound or a structural and functional analog or homolog thereof, wherein the chemical compound is identified as binding to and inhibiting the activation of the human MCH1 receptor by a process which comprises separately contacting cells expressing on their cell surface the human MCH1 receptor and producing a second messenger response upon activation of the human MCH1 receptor, wherein such cells do not normally express the human MCH1 receptor and the human MCH1 receptor is encoded by nucleic acid comprising the sequence shown in Figure 1 (Seq. ID No. 1) or contained in plasmid pEXJ.HR-TL231 (ATCC Accession No. 203197), with both the chemical compound and a second chemical compound known to activate the human MCH1 receptor, and with only the second chemical compound, under conditions suitable for activation of the human MCH1 receptor, and measuring the second messenger response in the presence of only the second chemical compound and in the presence of both the second chemical compound and the chemical compound, a smaller change in the second

messenger response in the presence of both the chemical compound and the second chemical compound than in the presence of only the second chemical compound indicating that the chemical compound inhibits activation of the 5 human MCH1 receptor, wherein the second chemical compound is MCH or a homolog or analog of MCH. In one embodiment, the second messenger response comprises chloride channel activation and the change in second messenger response is a smaller increase in the level of inward chloride current 10 in the presence of both the chemical compound and the second chemical compound than in the presence of only the second chemical compound.

This invention provides a process for preparing a 15 composition which comprises identifying a chemical compound which specifically binds to a human MCH1 receptor, and then admixing a carrier and the chemical compound or a structural and functional analog or homolog thereof, wherein the chemical compound is identified as binding to the human MCH1 receptor by a process involving 20 competitive binding which comprises contacting cells expressing on their cell surface the human MCH1 receptor, with both the chemical compound and a second chemical compound known to bind to the receptor, and separately with only the second chemical compound, under conditions 25 suitable for binding of both compounds, and detecting the extent of specific binding of the chemical compound to the human MCH1 receptor, a decrease in the binding of the second chemical compound to the human MCH1 receptor in the presence of the chemical compound indicating that the 30 chemical compound binds to the human MCH1 receptor, wherein the cells do not normally express the human MCH1 receptor, the human MCH1 receptor is encoded by nucleic acid comprising the sequence shown in Figure 1 (Seq. ID No. 1) or contained in plasmid pEXJ.HR-TL231 (ATCC 35

Accession No. 203197), and the second chemical compound is MCH or a homolog or analog of MCH.

This invention further provides a process for preparing a composition which comprises identifying a chemical compound which specifically binds to a human MCH1 receptor, and then admixing a carrier and the chemical compound or a structural and functional analog or homolog thereof, wherein the chemical compound is identified as binding to the human MCH1 receptor by a process involving competitive binding which comprises contacting a membrane preparation from cells expressing on their cell surface the human MCH1 receptor, with both the chemical compound and a second chemical compound known to bind to the receptor, and separately with only the second chemical compound, under conditions suitable for binding of both compounds, and detecting the extent of specific binding of the chemical compound to the human MCH1 receptor, a decrease in the binding of the second chemical compound to the human MCH1 receptor in the presence of the chemical compound indicating that the chemical compound binds to the human MCH1 receptor, wherein the cells do not normally express the human MCH1 receptor, the human MCH1 receptor is encoded by nucleic acid comprising the sequence shown in Figure 1 (Seq. ID No. 1) or contained in plasmid pEXJ.HR-TL231 (ATCC Accession No. 203197), and the second chemical compound is MCH or a homolog or analog of MCH.

This invention also provides a process for preparing a composition which comprises identifying a chemical compound which is a human MCH1 receptor antagonist, and then admixing a carrier and the chemical compound or a structural and functional analog or homolog thereof, wherein the chemical compound is identified as a human MCH1 receptor antagonist by a process which comprises

- contacting cells transfected with and expressing DNA encoding the human MCH1 receptor with the compound in the presence of a known human MCH1 receptor agonist, under conditions permitting the activation of the human MCH1 receptor, and detecting a decrease in human MCH1 receptor activity, so as to thereby determine whether the compound is a human MCH1 receptor antagonist, wherein the cells do not normally express the human MCH1 receptor, the human MCH1 receptor is encoded by nucleic acid comprising the sequence shown in Figure 1 (Seq. ID No. 1) or contained in plasmid pEXJ.HR-TL231 (ATCC Accession No. 203197), and the known human MCH1 receptor agonist is MCH or a homolog or analog of MCH.
- This invention still further provides a process for preparing a composition which comprises identifying a chemical compound which specifically binds to and inhibits the activation of a human MCH1 receptor, and then admixing a carrier and the chemical compound or a structural and functional analog or homolog thereof, wherein the chemical compound is identified as binding to and inhibiting activation of the human MCH1 receptor by a process which comprises separately contacting cells expressing on their cell surface the human MCH1 receptor and producing a second messenger response upon activation of the human MCH1 receptor, wherein such cells do not normally express the human MCH1 receptor and the human MCH1 receptor is encoded by nucleic acid comprising the sequence shown in Figure 1 (Seq. ID No. 1) or contained in plasmid pEXJ.HR-TL231 (ATCC Accession No. 203197), with both the chemical compound and a second chemical compound known to activate the human MCH1 receptor, and with only the second chemical compound, under conditions suitable for activation of the human MCH1 receptor, and measuring the second messenger response in the presence of only the second chemical

compound and in the presence of both the second chemical compound and the chemical compound, a smaller change in the second messenger response in the presence of both the chemical compound and the second chemical compound than in the presence of only the second chemical compound indicating that the chemical compound inhibits activation of the human MCH1 receptor, wherein the second chemical compound is MCH or a homolog or analog of MCH. In one embodiment, the second messenger response comprises chloride channel activation and the change in second messenger response is a smaller increase in the level of inward chloride current in the presence of both the chemical compound and the second chemical compound than in the presence of only the second chemical compound.

In one embodiment of any of the above methods, the cell is an insect cell. In another embodiment, the cell is a mammalian cell. In another embodiment, the mammalian cell is nonneuronal in origin. In further embodiments, the nonneuronal cell is a COS-7 cell, a 293 human embryonic kidney cell, a CHO cell, a NIH-3T3 cell, a mouse Y1 cell, or a LM(tk-) cell.

For the purposes of this invention, "antagonist potency" is measured as K_B which is defined as the equilibrium dissociation constant for the antagonist-receptor complex.

For the purposes of this invention, "agonist potency" is measured as EC₅₀ which is defined as the concentration that is required to elicit 50% of the maximum response in a functional assay.

Throughout the invention, the term "binding affinity" describes the concentration of a compound required to occupy one-half of the binding sites in a receptor

population, as detectable by radioligand binding. Binding affinity concentration can be represented as K_i , inhibition constant, or K_D , dissociation constant.

5 The term "selectivity of binding affinity" refers to the ability of a chemical compound to discriminate one receptor from another. For example, a compound showing selectivity for receptor A versus receptor B will bind receptor A at lower concentrations than those required to bind receptor B.
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Therefore, the statements of the form "binds to the MCH1 receptor with a binding affinity at least ten-fold higher than" a named receptor, indicates that the binding affinity at the MCH1 receptor is at least ten-fold greater than that for a named receptor, and binding affinity measurements (i.e. K_i or K_D) for the compound are at least ten-fold lower in numerical value.
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20 This invention provides a method of treating an eating disorder or obesity in a subject which comprises administering to the subject a therapeutically effective amount of an MCH1 antagonist which inhibits the activation of the MCH1 receptor. In an embodiment, the MCH1 antagonist additionally inhibits the activation of the MCH1 receptor with an antagonist potency which is at least 30-fold greater than the antagonist potency with which the MCH1 antagonist inhibits the activation of each of the 5-HT2C and MC-4 receptors.
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30 In a further embodiment, the MCH1 antagonist additionally inhibits the activation of the MCH1 receptor with an antagonist potency which is at least 10-fold greater than the antagonist potency with which the MCH1 antagonist inhibits the activation of each of the NPY1, NPY5, GALR1,
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5 GALR2, and GALR3 receptors. In another embodiment, the MCH1 antagonist additionally inhibits the activation of the MCH1 receptor with an antagonist potency which is at least 100-fold greater than the antagonist potency with which the MCH1 antagonist inhibits the activation of each of the 5-HT2C and MC-4 receptors.

10 In an additional embodiment, the MCH1 antagonist additionally inhibits the activation of the MCH1 receptor with an antagonist potency which is at least 100-fold greater than the antagonist potency with which the MCH1 antagonist inhibits the activation of each of the NPY1, NPY5, GALR1, GALR2, and GALR3 receptors. In an embodiment, the MCH1 antagonist additionally inhibits the activation of the MCH1 receptor with an antagonist potency which is at least 30-fold greater than the binding affinity with which the MCH1 antagonist binds to each of the 5-HT2C and MC-4 receptors.

15 20 In another embodiment, the MCH1 antagonist additionally inhibits the activation of the MCH1 receptor with an antagonist potency which is at least 10-fold greater than the binding affinity with which the MCH1 antagonist binds to each of the NPY1, NPY5, GALR1, GALR2, and GALR3 receptors. In a further embodiment, the MCH1 antagonist additionally inhibits the activation of the MCH1 receptor with an antagonist potency which is at least 100-fold greater than the binding affinity with which the MCH1 antagonist binds to each of the 5-HT2C and MC-4 receptors.

25 30 In an additional embodiment, the MCH1 antagonist additionally inhibits the activation of the MCH1 receptor with an antagonist potency which is at least 100-fold greater than the binding affinity with which the MCH1 antagonist binds to each of the NPY1, NPY5, GALR1, GALR2,

and GALR3 receptors. In yet another embodiment, the MCH1 antagonist additionally binds to the MCH1 receptor with a binding affinity which is at least 30-fold greater than the binding affinity with which the MCH1 antagonist binds to each of the 5-HT2C and MC-4 receptors. In still another embodiment, the MCH1 antagonist additionally binds to the MCH1 receptor with a binding affinity which is at least 10-fold greater than the binding affinity with which the MCH1 antagonist binds to each of the NPY1, NPY5, GALR1, GALR2, and GALR3 receptors.

In a further embodiment, the MCH1 antagonist additionally binds to the MCH1 receptor with a binding affinity which is at least 100-fold greater than the binding affinity with which the MCH1 antagonist binds to each of the 5-HT2C and MC-4 receptors. In an additional embodiment, the MCH1 antagonist additionally binds to the MCH1 receptor with a binding affinity which is at least 100-fold greater than the binding affinity with which the MCH1 antagonist binds to each of the NPY1, NPY5, GALR1, GALR2, and GALR3 receptors.

In another embodiment, the MCH1 antagonist additionally binds to the MCH1 receptor with a binding affinity which is at least 30-fold greater than the binding affinity with which the MCH1 antagonist binds to the dopamine D2 receptor. In another embodiment, the MCH1 antagonist additionally binds to the MCH1 receptor with a binding affinity which is at least 30-fold greater than the binding affinity with which the MCH1 antagonist binds to the histamine H1 receptor.

In still other embodiments, the MCH1 antagonist additionally binds to the MCH1 receptor with a binding affinity which is at least 100-fold greater than the

binding affinity with which the MCH1 antagonist binds the dopamine D2 receptor. In another embodiment, the MCH1 antagonist additionally binds to the MCH1 receptor with a binding affinity which is at least 100-fold greater than the binding affinity with which the MCH1 antagonist binds to the H1 histamine receptor.

In another embodiment, the MCH1 antagonist additionally binds to the MCH1 receptor with a binding affinity which is at least 200-fold greater than the binding affinity with which the MCH1 antagonist binds the dopamine D2 receptor. In still another embodiment, the MCH1 antagonist additionally binds to the MCH1 receptor with a binding affinity which is at least 200-fold greater than the binding affinity with which the MCH1 antagonist binds to the H1 histamine receptor.

In further embodiments, the MCH1 antagonist additionally binds to the MCH1 receptor with a binding affinity which is at least 10-fold greater than the binding affinity with which the MCH1 antagonist binds to the α_{1A} adrenoceptor. In another embodiment, the MCH1 antagonist additionally binds to the MCH1 receptor with a binding affinity which is at least 100-fold greater than the binding affinity with which the MCH1 antagonist binds to the α_{1A} adrenoceptor.

In other embodiments, the MCH1 antagonist additionally binds to the α_{1A} adrenoceptor with a binding affinity which is no more than 10-fold greater than the binding affinity with which the MCH1 antagonist binds to the MCH1 receptor. In still other embodiments, the MCH1 antagonist additionally binds to the α_{1A} adrenoceptor with a binding affinity which is no more than 100-fold greater than the binding affinity with which the MCH1 antagonist binds to the MCH1 receptor.

In any of the embodiments of the present invention, the eating or feeding disorder is bulimia, obesity or bulimia nervosa. In one embodiment, the subject is a vertebrate, a mammal, a human or a canine. In another embodiment, the MCH1 antagonist is administered in combination with food.

This invention also provides a method of treating an eating disorder in a subject which comprises administering to the subject a therapeutically effective amount of an MCH1 agonist which activates the MCH1 receptor. In one embodiment, the MCH1 agonist additionally activates the MCH1 receptor with an agonist potency which is at least 30-fold greater than the agonist potency with which the MCH1 agonist activates each of the 5-HT2C and MC-4 receptors.

In another embodiment, the MCH1 agonist additionally activates the MCH1 receptor with an agonist potency which is at least 10-fold greater than the agonist potency with which the MCH1 agonist activates each of the NPY1, NPY5, GALR1, GALR2, and GALR3 receptors. In a further embodiment, the MCH1 agonist additionally activates the MCH1 receptor with an agonist potency which is at least 100-fold greater than the agonist potency with which the MCH1 agonist activates each of the 5-HT2C and MC-4 receptors.

In yet another embodiment, the MCH1 agonist additionally activates the MCH1 receptor with an agonist potency which is at least 100-fold greater than the agonist potency with which the MCH1 agonist activates each of the NPY1, NPY5, GALR1, GALR2, and GALR3 receptors. In further embodiments, the eating disorder is anorexia nervosa. In another embodiment, the subject is a vertebrate, a mammal, a human or a canine. In a final embodiment, the MCH1

agonist is administered in combination with food.

In the subject invention a "therapeutically effective amount" is any amount of a compound which, when administered to a subject suffering from a disease against which the compounds are effective, causes reduction, remission, or regression of the disease. In the subject application, a "subject" is a vertebrate, a mammal, a human or a canine.

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This invention further provides a method of modifying feeding behavior of a subject which comprises administering to the subject an amount of a compound of the present invention effective to decrease the consumption of food by the subject and/or decrease the body mass of the subject. In one embodiment, the subject is a vertebrate, a mammal, a human or a canine. In another embodiment, the MCH1 antagonist is administered in combination with food.

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The present invention includes within its scope prodrugs of the compounds of the invention. In general, such prodrugs will be functional derivatives of the compounds of the invention which are readily convertible in vivo into the required compound. Thus, in the present invention, the term "administering" shall encompass the treatment of the various conditions described with the MCH1 antagonist specifically disclosed or with a compound which may not be specifically disclosed, but which converts to the specified MCH1 antagonist in vivo after administration to the patient. Conventional procedures for the selection and preparation of suitable prodrug derivatives are described, for example, in Design of Prodrugs, ed. H. Bundgaard, Elsevier, 1985.

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The present invention provides a method of treating depression and/or anxiety in a subject which comprises administering to the subject a composition comprising a pharmaceutically acceptable carrier and a therapeutically effective amount of a MCH1 antagonist, wherein:

- 5 (a) (1) the MCH1 antagonist does not inhibit the activity of central monoamine oxidase A greater than 50 percent, at a concentration of 10mM; and
10 (2) the MCH1 antagonist does not inhibit the activity of central monoamine oxidase B greater than 50 percent, at a concentration of 10mM; and
15 (b) the MCH1 antagonist binds to the MCH1 receptor with a binding affinity at least ten-fold higher than the binding affinity with which it binds to each of the following transporters: serotonin transporter, norepinephrine transporter, and dopamine transporter.

20 For the purposes of this invention the term "pharmaceutically acceptable carrier" has been defined herein.

25 In other embodiments, the MCH1 antagonist also binds to the MCH1 receptor with a binding affinity at least ten-fold higher than the binding affinity with which it binds to each of the human 5HT_{1A}, human 5HT_{1B}, human 5HT_{1D}, human 5HT_{1E}, human 5HT_{1F}, human 5HT_{2A}, rat 5HT_{2C}, human 5HT₄, human 5HT₆ and human 5HT₇ receptors.

30 In still another embodiment, the MCH1 antagonist also binds to the MCH1 receptor with a binding affinity at least ten-fold higher than the binding affinity with which it binds to the human histamine H₁ and H₂ receptors.

35 In still another embodiment, the MCH1 antagonist also

binds to the MCH1 receptor with a binding affinity at least ten-fold higher than the binding affinity with which it binds to the human dopamine D₁, D₂, D₃, D₄ and D₅ receptors.

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In a further embodiment, the MCH1 antagonist also binds to the MCH1 receptor with a binding affinity at least ten-fold higher than the binding affinity with which it binds to the human α_{1A} adrenoceptor, the human α_{1B} adrenoceptor and the human α_{1D} adrenoceptor.

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In another embodiment, the MCH1 antagonist also binds to the MCH1 receptor with a binding affinity at least ten-fold higher than the binding affinity with which it binds to the human α_{2A} adrenoceptor, the human α_{2B} adrenoceptor and the human α_{2C} adrenoceptor.

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In some embodiments the MCH1 antagonist does not inhibit the activity of central monoamine oxidase A greater than 60 percent. In further embodiments the MCH1 antagonist does not inhibit the activity of central monoamine oxidase B greater than 60 percent. In other embodiments the MCH1 antagonist does not inhibit the activity of central monoamine oxidase A greater than 70 percent. In still other embodiments the MCH1 antagonist does not inhibit the activity of central monoamine oxidase B greater than 70 percent.

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The binding properties of compounds at different receptors were determined using cultured cell lines that selectively express the receptor of interest. Cell lines were prepared by transfecting the cloned cDNA or cloned genomic DNA or constructs containing both genomic DNA and cDNA encoding the receptors as further described in the Experimental

A B P D E G E M T I C P D T

Details herein below. Furthermore, the binding interactions of compounds at different transporters and enzymes can be determined using tissue preparations and specific assays well known in the art.

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In connection with this invention, a number of cloned receptors discussed herein, as stably transfected cell lines, have been made pursuant to, and in satisfaction of, the Budapest Treaty on the International Recognition of 10 the Deposit of Microorganisms for the Purpose of Patent Procedure, and are made with the American Type Culture Collection, 10801 University Blvd., Manassas, Virginia 20110-2209. Specifically, these deposits have been accorded ATCC Accession Numbers as follows:

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ATCC Deposits:				
	Designation	Receptor	ATCC Accession No.	Date of Deposit
5		human GAL1	CRL-1650	
	(CHO) hGalR 2-264	human GAL2	CRL 12379	07/22/1997
	L-hGalR3-228	human GAL3	CRL-12373	07/01/1997
10	5HT1A-3	human 5-HT _{1A}	CRL 11889	05/11/1995
	Ltk-11	human 5-HT _{1B} (formerly human 5-HT1D2)	CRL 10422	04/17/1990
	Ltk-8-30-84	human 5-HT _{1D} (formerly human 5-HT1D1)	CRL 10421	04/17/1990
15	5HT _{1E} -7	human 5-HT _{1E}	CRL 10913	11/06/1991
	L-5-HT _{1F}	human 5-HT _{1F}	CRL 10957	12/27/1991
	L-NGC-5HT ₂	human 5-HT _{2A} (formerly human 5-HT2)	CRL 10287	10/31/1989
20	pSr-1c	rat 5-HT _{2C} (formerly rat 5HT1C)	67636	
	pBluescript-hS10	human 5-HT ₄	75392	12/22/1992
	L-5HT-4B	human 5-HT ₇ (formerly human 5-HT4B)	CRL 11166	10/20/1992
	L- α_{1C}	human α_{1A} (formerly human $\alpha 1C$)	CRL11140	09/25/1992

L- α_{1B}	human α_{1B}	CRL11139	09/25/1992
L- α_{1A}	human α_{1D} (formerly hum $\alpha 1A$)	CRL11138	09/25/1992
L- α_{2A}	human α_{2A}	CRL11180	11/06/1992
L-NGC- α_{2B}	human α_{2B}	CRL10275	10/25/1989
L- α_{2C}	human α_{2C}	CRL11181	11/06/1992
pDopD ₁ -GL-30	human D ₅ (formerly hum D1 β)	40839	07/10/1990
pCEXV-H ₁	human H ₁	75346	11/06/1992

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- The "5-HT_{1C}", "5-HT_{1D1}", "5-HT_{1D2}", "5-HT_{4B}", and "5-HT₂" receptors were renamed the "5-HT_{2C}", "5-HT_{1D}", "5-HT_{1B}", "5-HT₇", and "5-HT_{2A}" receptors, respectively, by the Serotonin Receptor Nomenclature Committee of the IUPHAR.
- The "human α_{1C} ", "human α_{1A} ", and "human D_{1 β} " were renamed the "human α_{1A} ", "human α_{1D} ", and "human D₅" respectively.

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The following receptor sequences have been deposited with the GenBank DNA database, which is managed by the National Center for Biotechnology (Bethesda, MD).

GENBANK DEPOSITS		
DESIGNATION	RECEPTOR	GENBANK No.
5 human mRNA for D-1 receptor	human D ₁ (formerly human D _{1α})	X58987
10 human dopamine D2 receptor (DRD2) mRNA complete cds	human D ₂	M29066
15 Rat mRNA for dopamine D3 receptor	rat D ₃	X53944
20 Homo sapiens dopamine D4 receptor (DRD4) gene (D4.4) sequence	human D ₄	L12397

* The "human D_{1α}" receptor was renamed the "human D₁" receptor.

Thus, once the gene for a targeted receptor subtype is cloned, it is placed into a recipient cell which then expresses the targeted receptor subtype on its surface. This cell, which expresses a single population of the targeted human receptor subtype, is then propagated resulting in the establishment of a cell line. This cell line, which constitutes a drug discovery system, is used in two different types of assays: binding assays and functional assays. In binding assays, the affinity of a compound for both the receptor subtype that is the target

of a particular drug discovery program and other receptor subtypes that could be associated with side effects are measured. These measurements enable one to predict the potency of a compound, as well as the degree of selectivity that the compound has for the targeted receptor subtype over other receptor subtypes. The data obtained from binding assays also enable chemists to design compounds toward or away from one or more of the relevant subtypes, as appropriate, for optimal therapeutic efficacy. In functional assays, the nature of the response of the receptor subtype to the compound is determined. Data from the functional assays show whether the compound is acting to inhibit or enhance the activity of the receptor subtype, thus enabling pharmacologists to evaluate compounds rapidly at their ultimate human receptor subtypes targets permitting chemists to rationally design drugs that will be more effective and have fewer or substantially less severe side effects than existing drugs.

Approaches to designing and synthesizing receptor subtype-selective compounds are well known and include traditional medicinal chemistry and the newer technology of combinatorial chemistry, both of which are supported by computer-assisted molecular modeling. With such approaches, chemists and pharmacologists use their knowledge of the structures of the targeted receptor subtype and compounds determined to bind and/or activate or inhibit activation of the receptor subtype to design and synthesize structures that will have activity at these receptor subtypes.

Combinatorial chemistry involves automated synthesis of a variety of novel compounds by assembling them using different combinations of chemical building blocks. The

- use of combinatorial chemistry greatly accelerates the process of generating compounds. The resulting arrays of compounds are called libraries and are used to screen for compounds ("lead compounds") that demonstrate a sufficient level of activity at receptors of interest. Using combinatorial chemistry it is possible to synthesize "focused" libraries of compounds anticipated to be highly biased toward the receptor target of interest.
- Once lead compounds are identified, whether through the use of combinatorial chemistry or traditional medicinal chemistry or otherwise, a variety of homologs and analogs are prepared to facilitate an understanding of the relationship between chemical structure and biological or functional activity. These studies define structure activity relationships which are then used to design drugs with improved potency, selectivity and pharmacokinetic properties. Combinatorial chemistry is also used to rapidly generate a variety of structures for lead optimization. Traditional medicinal chemistry, which involves the synthesis of compounds one at a time, is also used for further refinement and to generate compounds not accessible by automated techniques. Once such drugs are defined the production is scaled up using standard chemical manufacturing methodologies utilized throughout the pharmaceutical and chemistry industry.
- This invention will be better understood from the Experimental Details which follow. However, one skilled in the art will readily appreciate that the specific methods and results discussed are merely illustrative of the invention as described more fully in the claims which follow thereafter.

EXPERIMENTAL DETAILS

Materials and methods

5 Cloning of human MCH1 receptor

Discovery of an Expressed Sequence Tag (EST) F07228 in GENEMBL Homologous to FB41a

A BLAST search of GENEMBL was performed with the GCG sequence analysis package (Genetics Computer Group, Madison, WI) using a Synaptic Pharmaceutical Corporation proprietary sequence, FB41a, as a query. This resulted in the identification of an EST (accession number F07228) with a high degree of homology to FB41a and somatostatin, opiate and galanin receptors.

15 Construction and Screening of a Human Hippocampal cDNA Library

Poly A+ RNA was purified from human hippocampal RNA (Clontech) using a FastTrack kit (Invitrogen, Corp.). DS-cDNA was synthesized from poly A+ RNA according to Gubler and Hoffman (1983) with minor modifications. The resulting cDNA was ligated to BstXI adaptors (Invitrogen, Corp.) and the excess adaptors removed by exclusion column chromatography. High molecular weight fractions of size-selected ds-cDNA were ligated in pEXJ.BS, an Okayama and Berg expression vector modified from pcEXV (Miller and Germain, 1986) to contain BstXI and other additional restriction sites. A total of 2.2×10^6 independent clones with a mean insert size of 3.0 kb were generated. The library was plated on agar plates (ampicillin selection) and glycerol stocks for 450 pools of 5000 independent clones were prepared. Primary glycerol stocks were also grouped together in groups of approximately 10 to create superpools.

Cloning of the full-length sequence of MCH1

Glycerol stocks of the superpools and primary pools from the human hippocampal cDNA library were screened by PCR with F07228 specific primers T579 and T580 using Taq DNA Polymerase (Boehringer-Mannheim, Indianapolis, IN) and the following PCR protocol: 94°C hold for 5 minutes; 40 cycles of 94°C for 2 minute, 68°C for 4 minutes; 7 minute hold at 68°C; 4°C hold until the samples are run on a gel. One positive primary pool 490, was successively divided into subpools, amplified in LB medium overnight and screened by PCR using primers T579 and T580. One positive subpool, 490-4-10-23 was plated on agar plates (ampicillin selection), and colonies were transferred to nitrocellulose membranes (Schleicher and Schuell, Keene, NH). Filters were hybridized for two days under high stringency conditions with 10⁶ cpm/ml of a ³²P-labeled cDNA probe, T581, designed against the F07228 EST sequence. Filters were washed and apposed to Biomax MS film (Kodak). Seven positive colonies were picked, streaked on LB-AMP plates, and grown overnight. Two individual colonies from each of the original seven were picked and subjected to vector-anchored PCR using the following primer pairs: T95, T580 and T94, T579. One positive colony, G1, was amplified overnight in TB and processed for plasmid purification. This plasmid was designated TL230 and sequenced on both strands with a Sequenase kit (US Biochemical, Cleveland, Ohio). Nucleotide and peptide sequence analysis were performed with GCG programs (Genetics Computer Group, Madison, WI). A *Hind*III- *Kpn*I fragment of TL230 was subcloned into the mammalian expression vector pEXJ, and named TL231.

Primers and Probes:

TL579: 5'-GGGAACTCCACGGTCATCTTCGCGGT-3' (SEQ ID NO: 5)

TL580: 5'-TAGCGGTCAATGGCCATGGCGGTCAG-3' (SEQ ID NO: 6)

TL581:

5'-CTCCTGGGCATGCCCTTCATGATCCACCAGCTCATGGCAATGGG-3'

(SEQ ID NO: 7)

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TL94: 5'-CTTCTAGGCCTGTACGGAAGTGTAA-3' (SEQ ID NO: 8)

TL95: 5'-GTTGTGGTTGTCCAAACTCATCAATG-3' (SEQ ID NO: 9)

10 Isolation of a Fragment of a species homologue of TL231
(human MCH1)

To obtain a fragment of a species homologue of TL231, the species genomic DNA (Clontech) may be amplified with a forward PCR primer corresponding to one of the TM regions of TL231 and a reverse primer corresponding to another TM region of TL231. PCR may be performed with the Expand Long Template PCR System (Boeringer Mannheim), for example, under the following conditions: 30 sec at 94°C, 1.5 min at 50°C, 1.5 min at 68 °C for 40 cycles, with a pre- and post-incubation of 5 min at 94°C and 7 min at 68°C, respectively. A band is isolated, subcloned using the TA cloning kit (Invitrogen), and sequenced. The sequence is run and analyzed on an ABI PRISM 377 BigDye Terminator Cycle Sequencing Kit Sequencer. Forward and reverse PCR primers are designed against this sequence and used to amplify a band from genomic DNA using, for example, the following conditions: 30 sec at 94°C, 1.5 min at 68°C for 35 cycles, with a pre- and post-incubation of 5 min at 94°C and 5 min at 68°C, respectively. The PCR product is subcloned using the TA cloning kit (Invitrogen). Miniprep cultures of transformants are prepared and sequenced as above.

35 Isolation of a full-length species homolog of TL231 (human

MCH1)

A nucleic acid sequence encoding an MCH1 receptor may be isolated using standard molecular biology techniques and approaches such as those briefly described below:

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Approach #1: To obtain a full-length MCH1 receptor, a cosmid library could be screened with a ³²P-labeled oligonucleotide probe.

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The full-length sequence may be obtained by sequencing this cosmid clone with additional sequencing primers. Since one intron is present in this gene the full-length intronless gene may be obtained from cDNA using standard molecular biology techniques. For example, a forward PCR primer designed in the 5'UT and a reverse PCR primer designed in the 3'UT may be used to amplify a full-length, intronless gene from cDNA. Standard molecular biology techniques could be used to subclone this gene into a mammalian expression vector.

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Approach #2: Standard molecular biology techniques could be used to screen commercial cDNA phage libraries by hybridization under high stringency with a ³²P-labeled oligonucleotide probe. One may isolate a full-length MCH1 receptor by obtaining a plaque purified clone from the lambda libraries and then subjecting the clone to direct DNA sequencing. Alternatively, standard molecular biology techniques could be used to screen in-house cDNA plasmid libraries by PCR amplification of library pools using primers to the MCH1 sequence. A full-length clone could be isolated by Southern hybridization of colony lifts of positive pools with a ³²P-labeled oligonucleotide probe.

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Approach #3: As yet another alternative method, one could utilize 3' and 5' RACE to generate PCR products from cDNA

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expressing MCH1 which contain the additional sequences of MCH1. These RACE PCR products could then be sequenced to determine the missing sequence. This new sequence could then be used to design a forward PCR primer in the 5'UT and a reverse primer in the 3'UT. These primers could then be used to amplify a full-length MCH1 clone from cDNA.

Construction of Human MCH1 Mutants

The plasmid TL231 encodes three in frame methionine residues, any of which could potentially initiate translation of the MCH1 receptor. The ability of these residues to function in a heterologous expression system was examined by constructing mutants of TL231 in which one or more of the downstream methionine residues was mutated to alanine. Mutagenesis was performed using the QuickChange site-directed mutagenesis kit (Stratagene). Each 50 ul PCR reaction contained 10 mM KCl, 10 mM $(\text{NH}_4)_2\text{SO}_4$, 20 mM Tris-HCl (pH 8.8), 2 mM MgSO₄, 0.1% Triton X-100, 0.1mg/ml nuclease-free BSA, 114 ng each of two mutagenesis primers (see below), 50 ng of plasmid DNA template (see below), 2.5 units of PfuTurbo DNA polymerase, and 1 ul of the proprietary dNTP mix provided in the kit. Thermocycling was performed with an Applied Biosystems 9700 machine using the following cycling parameters: one cycle of 95° for 30 seconds; eighteen cycles of 95° for 30 seconds, 55° for 1 minute, 68° for 2.5 minutes; a final hold at 4°. Next, 1 ul (10 units) of DpnI restriction enzyme was added to the mutagenesis reaction followed by incubation at 37° for 1 hour. A 2 ul aliquot of this digestion was used to transform 50 ul of E.coli XL1-Blue cells provided with the mutagenesis kit. Transformants were selected by their ability to grow at 37° on LB plates containing 100 ug/ml ampicillin. Single

colonies which resulted from the overnight incubation of the plates were used to inoculate 2 ml cultures of LB-ampicillin and allowed to grow overnight at 37° with shaking. Miniprep DNA was prepared from these cultures 5 using the Qiagen miniprep system and subjected to automated sequence analysis. This allowed both the confirmation of the desired mutation and the integrity of the remainder of the MCH1 coding sequence. After identification of a correctly mutated clone, a large scale 10 DNA prep was prepared using a Qiagen megaprep column.

To create the clone encoding only the M70A mutation, the template DNA was TL231 and the mutagenesis primers were RP192 and RP193. This clone is designated R106 (SEQ ID NO: 16) and encodes only the first two potential start codons 15 (See Figure 12). To create the clone encoding both the M6A and the M70A mutations, the template DNA was R106 and the mutagenesis primers were RP190 and RP191. The resulting clone is designated R114 (SEQ ID NO: 17) and encodes only 20 first start codon (See Figure 12).

If desired, the same mutagenesis technology can be employed to construct additional MCH1 mutants that encode other combinations of the available methionine residues. 25 The mutation M1A could be constructed using primers X1 and X2. Such a change would eliminate the first methionine but retain the two downstream residues. Likewise, the double mutation M1A, M70A could be constructed by sequentially using primer pairs X1/ X2 and RP192/RP193. 30 This would create a gene in which only the second methionine was left intact.

Primers used in the generation of hMCH1 mutant receptor constructs:

	<u>Mutant</u>	<u>Primer</u>	<u>Primer Sequence</u>
	R106	RP192	5' CGGCAC TGGCTGGCGGACCTGGAAGCCTCG 3' (SEQ ID NO: 18)
5	M70A)	RP193	5' CGAGGCTTCCAGGTCCGCCAGCCAGTGCCG 3' (SEQ ID NO: 19)
	R114	RP190	5' ATGTCAGTGGAGCCCGAAGAAGGGAGTGGG 3' (SEQ ID NO: 20)
10	(M6A,	RP191	5' CCCACTCCCTTCTCGCGCTCCCAGTGACAT 3'
	M70A)	(SEQ ID NO: 21)	
	(M1A)	X1	5' TAATGTGTCTAGGTGGCGTCAGTGGGAGCCATG 3'
15	(SEQ ID NO: 22)	X2	5' CATGGCTCCCACTGACGCCACCTAGACACATTA 3'
			(SEQ ID NO: 23)

Construction of a short form of the human MCH1 receptor

20 A short form of the human MCH1 receptor expressing only the most downstream of the three potential initiating methionines was generated as follows. TL231 was amplified with BB1122 (a forward primer beginning 10 nucleotides upstream of the third methionine in TL231, and also incorporating a *Hind*III site) and BB1123 (a reverse primer in the second transmembrane domain) and the resulting product digested with *Hind*III and *Bgl*IIA. PCR was performed with the Expand Long Template PCR System (Roche Molecular Biochemicals, Indianapolis, IN) under the following conditions: 20 seconds at 94°C, 1 minute at 68°C for 40 cycles, with a pre- and post-incubation of 5 minutes at 94°C and 7 minutes at 68°C respectively. The 270 bp product was gel purified and ligated to a 4 kb

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HindIII/BglII restriction fragment from TL231. The resulting construct was named BO120.

5 Primers used in the construction of the truncated human MCH1 receptor:

BB1122 5' - TGACACTAAGCTTCACTGGCTGGATGGACCTGGAAGC -3' (SEQ ID NO: 24)

10 BB1123 5' - GCCCAGGAGAAAGAGGAGATCTAC -3' (SEQ ID NO: 25)

Host cells

15 A broad variety of host cells can be used to study heterologously expressed proteins. These cells include but are not restricted to assorted mammalian lines such as; Cos-7, CHO, LM(tk-), HEK293, etc.; insect cell lines such as; Sf9, Sf21, etc.; amphibian cells such as xenopus oocytes; and others.

20 COS-7 cells are grown on 150 mm plates in DMEM with supplements (Dulbecco's Modified Eagle Medium with 10% bovine calf serum, 4 mM glutamine, 100 units/ml penicillin/100 µg/ml streptomycin) at 37°C, 5% CO₂. Stock plates of COS-7 cells are trypsinized and split 1:6 every 3-4 days.

30 Human embryonic kidney 293 cells are grown on 150 mm plates in DMEM with supplements (10% bovine calf serum, 4 mM glutamine, 100 units/ml penicillin/100 µg/ml streptomycin) at 37°C, 5% CO₂. Stock plates of 293 cells are trypsinized and split 1:6 every 3-4 days.

35 Mouse fibroblast LM(tk-) cells are grown on 150 mm plates in D-MEM with supplements (Dulbecco's Modified Eagle

Medium with 10% bovine calf serum, 4 mM glutamine, 100 units/ml penicillin/100 µg/ml streptomycin) at 37°C, 5% CO₂. Stock plates of LM(tk-) cells are trypsinized and split 1:10 every 3-4 days.

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Chinese hamster ovary (CHO) cells were grown on 150 mm plates in HAM's F-12 medium with supplements (10% bovine calf serum, 4 mM L-glutamine and 100 units/ml penicillin/100 µg/ml streptomycin) at 37°C, 5% CO₂. Stock plates of CHO cells are trypsinized and split 1:8 every 3-4 days.

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Mouse embryonic fibroblast NIH-3T3 cells are grown on 150 mm plates in Dulbecco's Modified Eagle Medium (DMEM) with supplements (10% bovine calf serum, 4 mM glutamine, 100 units/ml penicillin/100 µg/ml streptomycin) at 37°C, 5% CO₂. Stock plates of NIH-3T3 cells are trypsinized and split 1:15 every 3-4 days.

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Sf9 and Sf21 cells are grown in monolayers on 150 mm tissue culture dishes in TMN-FH media supplemented with 10% fetal calf serum, at 27°C, no CO₂. High Five insect cells are grown on 150 mm tissue culture dishes in Ex-Cell 400™ medium supplemented with L-Glutamine, also at 27°C, no CO₂.

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In some cases, cell lines that grow as adherent monolayers can be converted to suspension culture to increase cell yield and provide large batches of uniform assay material for routine receptor screening projects.

Xenopus oocytes can also be used as a host system for transient expression of heterologous proteins. Their maintenance and usage is described in the electrophysiological methods section that follows.

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Transient expression

DNA encoding proteins to be studied can be transiently expressed in a variety of mammalian, insect, amphibian and other cell lines by several methods including but not restricted to; calcium phosphate-mediated, DEAE-dextran mediated, Liposomal-mediated, viral-mediated, electroporation-mediated and microinjection delivery. Each of these methods may require optimization of assorted experimental parameters depending on the DNA, cell line, and the type of assay to be subsequently employed.

A typical protocol for the calcium phosphate method as applied to LM(tk-) cells is described as follows; Adherent cells are harvested approximately twenty-four hours before transfection and replated at a density of $1\text{-}2 \times 10^5$ cells/cm² in a 100 mm tissue culture dish and allowed to incubate over night at 37°C at 5% CO₂. 250 μl of a mixture of CaCl₂ and DNA (20 μg DNA in 250 mM CaCl₂) is added to a 5 ml plastic tube and 250 μl of 2X HBS (250 mM NaCl, 10 mM KCl, 1.5 mM Na₂HPO₄, 12 mM dextrose, 50 mM HEPES) is slowly added with gentle mixing. The mixture is allowed to incubate for 20 minutes at room temperature to allow a DNA precipitate to form. The cells are then washed with complete medium, 10 ml of culture medium is added to each plate, followed by addition of the DNA precipitate. The cells are then incubated for 24 to 48 hours at 37°C at 5% CO₂.

A typical protocol for the DEAE-dextran method as applied to Cos-7 cells is described as follows; Cells to be used for transfection are split 24 hours prior to the transfection to provide flasks which are 70-80% confluent at the time of transfection. Briefly, 8 μg of receptor DNA plus 8 μg of any additional DNA needed (e.g. G_a protein expression vector, reporter construct, antibiotic

resistance marker, mock vector, etc.) are added to 9 ml of complete DMEM plus DEAE-dextran mixture (10 mg/ml in PBS). Cos-7 cells plated into a T225 flask (sub-confluent) are washed once with PBS and the DNA mixture is added to each flask. The cells are allowed to incubate for 30 minutes at 37°C, 5% CO₂. Following the incubation, 36 ml of complete DMEM with 80 µM chloroquine is added to each flask and allowed to incubate an additional 3 hours. The medium is then aspirated and 24 ml of complete medium containing 10% DMSO for exactly 2 minutes and then aspirated. The cells are then washed 2 times with PBS and 30 ml of complete DMEM added to each flask. The cells are then allowed to incubate over night. The next day the cells are harvested by trypsinization and reseeded as needed depending upon the type of assay to be performed.

A typical protocol for liposomal-mediated transfection as applied to CHO cells is described as follows; Cells to be used for transfection are split 24 hours prior to the transfection to provide flasks which are 70-80% confluent at the time of transfection. A total of 10µg of DNA which may include varying ratios of receptor DNA plus any additional DNA needed (e.g. G_a protein expression vector, reporter construct, antibiotic resistance marker, mock vector, etc.) is used to transfect each 75 cm² flask of cells. Liposomal mediated transfection is carried out according to the manufacturer's recommendations (LipofectAMINE, GibcoBRL, Bethesda, MD). Transfected cells are harvested 24 h post transfection and used or reseeded according the requirements of the assay to be employed.

A typical protocol for the electroporation method as applied to Cos-7 cells is described as follows; Cells to be used for transfection are split 24 hours prior to the

transfection to provide flasks which are subconfluent at the time of transfection. The cells are harvested by trypsinization resuspended in their growth media and counted. 4×10^6 cells are suspended in 300 μl of DMEM and placed into an electroporation cuvette. 8 μg of receptor DNA plus 8 μg of any additional DNA needed (e.g. G_α protein expression vector, reporter construct, antibiotic resistance marker, mock vector, etc.) is added to the cell suspension, the cuvette is placed into a BioRad Gene Pulser and subjected to an electrical pulse (Gene Pulser settings: 0.25 kV voltage, 950 μF capacitance). Following the pulse, 800 μl of complete DMEM is added to each cuvette and the suspension transferred to a sterile tube. Complete medium is added to each tube to bring the final cell concentration to 1×10^5 cells/100 μl . The cells are then plated as needed depending upon the type of assay to be performed.

A typical protocol for viral mediated expression of heterologous proteins is described as follows for baculovirus infection of insect Sf9 cells. The coding region of DNA encoding the receptor disclosed herein may be subcloned into pBlueBacIII into existing restriction sites or sites engineered into sequences 5' and 3' to the coding region of the polypeptides. To generate baculovirus, 0.5 μg of viral DNA (BaculoGold) and 3 μg of DNA construct encoding a polypeptide may be co-transfected into 2×10^6 *Spodoptera frugiperda* insect Sf9 cells by the calcium phosphate co-precipitation method, as outlined in by Pharmingen (in "Baculovirus Expression Vector System: Procedures and Methods Manual"). The cells then are incubated for 5 days at 27°C. The supernatant of the co-transfection plate may be collected by centrifugation and the recombinant virus plaque purified. The procedure to infect cells with virus, to prepare stocks of virus and to

titer the virus stocks are as described in Pharmingen's manual. Similar principals would in general apply to mammalian cell expression via retro-viruses, Simliki forest virus and double stranded DNA viruses such as adeno-, herpes-, and vaccinia-viruses, and the like.

5 Microinjection of cRNA encoding for proteins of interest is useful for the study of protein function in xenopus oocytes as well as cultured mammalian cells. A typical 10 protocol for the preparation of cRNA and injection into xenopus oocytes can be found in the following electrophysiology section.

Stable expression

15 Heterologous DNA can be stably incorporated into host cells, causing the cell to perpetually express a foreign protein. Methods for the delivery of the DNA into the cell are similar to those described above for transient expression but require the co-transfection of an ancillary 20 gene to confer drug resistance on the targeted host cell. The ensuing drug resistance can be exploited to select and maintain cells that have taken up the heterologous DNA. An assortment of resistance genes are available including but not restricted to Neomycin, Kanamycin, and Hygromycin. For 25 the purposes of receptor studies, stable expression of a heterologous receptor protein is carried out in, but not necessarily restricted to, mammalian cells including, CHO, HEK293, LM(tk-), etc.

30 **Cell membrane preparation**

For binding assays, pellets of transfected cells are suspended in ice-cold buffer (20 mM Tris.HCl, 5 mM EDTA, pH 7.4) and homogenized by sonication for 7 sec. The cell lysates are centrifuged at 200 x g for 5 min at 4°C. The 35 supernatants are then centrifuged at 40,000 x g for 20 min

at 4°C. The resulting pellets are washed once in the homogenization buffer and suspended in binding buffer (see methods for radioligand binding). Protein concentrations are determined by the method of Bradford (1976) using bovine serum albumin as the standard. Binding assays are usually performed immediately, however it is possible to prepare membranes in batch and store frozen in liquid nitrogen for future use.

10 Radioligand binding assays

Cells may be screened for the presence of endogenous human receptor by radioligand binding (described in detail below). Cells with either no or a low level of the endogenous human receptor disclosed herein may be transfected with the exogenous receptor.

15 MCH1 binding experiments with membranes (20-40 µg membrane protein) from transfected cells are performed with 0.1 nM [¹²⁵I]Phe¹³-Tyr¹⁹-MCH (Custom labeled by NEN) using 20 incubation buffer consisting of 50mM Tris pH 7.4, 10mM MgCl₂, 2 µg/ml aprotonin, 0.5mM PMSF and 50 µg/ml bacitracin. Binding is performed at 25°C for 1 hr. Incubations are terminated by rapid vacuum filtration over 25 GF/C glass fiber filters, presoaked in 5% PEI using 50 mM Tris pH 7.4 containing 0.01% triton X-100 as wash buffer. In all experiments nonspecific binding is defined using 10 µM unlabeled MCH.

30 Functional assays

Cells may be screened for the presence of endogenous mammalian receptor using functional assays (described in detail below). Cells with no or a low level of endogenous receptor present may be transfected with the exogenous receptor for use in the following functional assays.

A wide spectrum of assays can be employed to screen for receptor activation. These range from traditional measurements of phosphatidyl inositol, cAMP, Ca⁺⁺, and K⁺, for example; to systems measuring these same second messengers but which have been modified or adapted to be higher throughput, more generic, and more sensitive; to cell based platforms reporting more general cellular events resulting from receptor activation such as metabolic changes, differentiation, and cell division/proliferation, for example; to high level organism assays which monitor complex physiological or behavioral changes thought to be involved with receptor activation including cardiovascular, analgesic, orexigenic, anxiolytic, and sedation effects, for example.

15

Cyclic AMP (cAMP) assay

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The receptor-mediated stimulation or inhibition of cyclic AMP (cAMP) formation may be assayed in cells expressing the mammalian receptors. Cells are plated in 96-well plates and incubated in Dulbecco's phosphate buffered saline (PBS) supplemented with 10 mM HEPES, 1mM isobutylmethylxanthine for 20 min at 37°C, in 5% CO₂. Test compounds are added with or without 10 µM forskolin and incubated for an additional 10 min at 37°C. The medium is then aspirated and the reaction stopped by the addition of 100 mM HCl. The plates are stored at 4°C for 15 min, and the cAMP content in the stopping solution measured by radioimmunoassay. Radioactivity may be quantified using a gamma counter equipped with data reduction software.

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Arachidonic acid release assay

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Cells expressing the mammalian receptor are seeded into 96 well plates and grown for 3 days in HAM's F-12 with supplements. [³H]-arachidonic acid (specific activity = 0.75 µCi/ml) is delivered as a 100 µL aliquot to each well

and samples were incubated at 37° C, 5% CO₂ for 18 hours. The labeled cells are washed three times with 200 μL HAM's F-12. The wells are then filled with medium (200 μL) and the assay is initiated with the addition of peptides or buffer (22 μL). Cells are incubated for 30 min at 37°C, 5% CO₂. Supernatants are transferred to a microtiter plate and evaporated to dryness at 75°C in a vacuum oven. Samples are then dissolved and resuspended in 25 μL distilled water. Scintillant (300 μL) is added to each well and samples are counted for ³H in a Trilux plate reader. Data are analyzed using nonlinear regression and statistical techniques available in the GraphPAD Prism package (San Diego, CA).

15 Intracellular calcium mobilization assay

The intracellular free calcium concentration may be measured by microspectrofluorometry using the fluorescent indicator dye Fura-2/AM (Bush et al, 1991). Cells are seeded onto a 35 mm culture dish containing a glass coverslip insert, washed with HBS and loaded with 100 μL of Fura-2/AM (10 μM) for 20 to 40 min. After washing with HBS to remove the Fura-2/AM solution, cells are equilibrated in HBS for 10 to 20 min. Cells are then visualized under the 40X objective of a Leitz Fluovert FS microscope and fluorescence emission is determined at 510 nM with excitation wavelengths alternating between 340 nM and 380 nM. Raw fluorescence data are converted to calcium concentrations using standard calcium concentration curves and software analysis techniques.

30

Inositol phosphate assay

Guidelines for cell preparation and assay of the second messenger inositol phosphate (IP) are described below for a typical protocol involving transiently transfected Cos-7 cells; For a 96 well microplate format assay, cells are

plated at 70,000 cells per well and allowed to incubate for 24 hours after the transfection procedure. The cells are then labeled with 0.5 μ Ci [3 H]myo-inositol per micro-well over night at 37°C, 5% CO₂. Immediately before the assay, the medium is removed and replaced with 90 μ l PBS containing 10 mM LiCl. The plates are then incubated for 15 minutes at 37°C, 5% CO₂. Following the incubation, the transfectants are challenged with agonist (10 μ l/well; 10X concentration) for 30 minutes at 37°C, 5% CO₂. The challenge is terminated and the cells lysed by the addition of 100 μ l cold 5% v/v trichloroacetic acid (TCA), followed by an incubation at 4°C for greater than 30 minutes. Total IPs are isolated from the lysate by ion exchange chromatography. Briefly, the lysed contents of the wells are transferred to a Multiscreen HV filter plate (Millipore) containing 100 μ l Dowex AG1-X8 suspension (50% v/v, water:resin) (200-400 mesh, formate form). The filter plates are placed on a vacuum manifold to wash and elute the resin bed. Each well is first washed 2 times with 200 μ l 5 mM myoinositol. Total [3 H]IPs are eluted with 75 μ l of 1.2 M ammonium formate/0.1 M formic acid into Wallac 96-well plates. 200 μ l of SuperMix scintillation cocktail is added to each well, mixed well, allowed to equilibrate and counted on a Micro Beta Trilux scintillation counter. (Note: The assay may be scaled to a 24 well format by simple adjustment of reagent volumes and employing individual chromatographic columns.)

GTP γ S functional assay

Membranes from cells transfected with the mammalian receptors are suspended in assay buffer (50 mM Tris, 100 mM NaCl, 5 mM MgCl₂, pH 7.4) supplemented with 0.2% BSA and 10 μ M GDP. Membranes are incubated on ice for 20 minutes, transferred to a 96-well Millipore microtiter GF/C filter plate and mixed with GTP γ ³⁵S (e.g., 250,000

cpm/sample, specific activity ~1000 Ci/mmol) plus or minus GTP γ S (final concentration = 100 μ M). Final membrane protein concentration \approx 90 μ g/ml. Samples are incubated in the presence or absence of MCH (final concentration = 1 μ M) for 30 min. at room temperature, then filtered on a Millipore vacuum manifold and washed three times with cold assay buffer. Samples collected in the filter plate are treated with scintillant and counted for 35 S in a Trilux (Wallac) liquid scintillation counter. It is expected that optimal results are obtained when the mammalian receptor membrane preparation is derived from an appropriately engineered heterologous expression system, i.e., an expression system resulting in high levels of expression of the mammalian receptor and/or expressing G-proteins having high turnover rates (for the exchange of GDP for GTP). GTP γ S assays are well-known in the art, and it is expected that variations on the method described above, such as are described by e.g., Tian et al. (1994) or Lazareno and Birdsall (1993), may be used by one of ordinary skill in the art.

Transcription assay

Guidelines for cell preparation and assay of receptor mediated transcription of Cos-7 cells transiently transfected by the DEAE-dextran method in a 96 microwell format is as follows; The c-fos- β -gal promoter/reporter construct used for these studies consists of the cfos promoter region (-384 to +19) (Schilling et al 1991, Yalkinoglu et al, 1995) inserted upstream of β -galactosidase cDNA containing expression vector pNASS β (Clontech). Transcription activity is measured by assay of β -galactosidase enzyme activity as detected in a colorimetric assay. Forty-eight hours following transient transfection, the medium is removed and replaced with medium containing drug (e.g. MCH) typically at a

concentration of 10 μ M. The cells are allowed to incubate at 37°C, 5% CO₂ for at least 18 hours, after which the medium is aspirated and the cells washed with 200 μ l PBS/well. The cells are then lysed with 100 μ l AB buffer (100 mM Sodium Phosphate buffer, pH 8.0, 2 mM MgSO₄, 0.1 mM MnCl₂) for 10 minutes at room temperature. 100 μ l of AB/Tx/ β -mercaptoethanol (AB buffer with 0.5% Triton X-100, 40 mM β -mercaptoethanol) is then added to each well and the lysate allowed to incubate an additional 10 minutes at room temperature. The enzymatic color reaction is initiated by the addition of the substrate, ONPG/AB (4 mg/ml O-nitrophenyl- β -D-galactopyranoside in AB buffer). The reaction is allowed to proceed for 30 minutes or until yellow color becomes evident. Measurement of optical density is taken at 405 nm using a Dynatech microplate reader.

MAP kinase assay

MAP kinase (mitogen activated kinase) may be monitored to evaluate receptor activation. MAP kinase is activated by multiple pathways in the cell. A primary mode of activation involves the ras/raf/MEK/MAP kinase pathway. Growth factor (tyrosine kinase) receptors feed into this pathway via SHC/Grb-2/SOS/ras. Gi coupled receptors are also known to activate ras and subsequently produce an activation of MAP kinase. Receptors that activate phospholipase C (Gq and G11) produce diacylglycerol (DAG) as a consequence of phosphatidyl inositol hydrolysis. DAG activates protein kinase C which in turn phosphorylates MAP kinase.

MAP kinase activation can be detected by several approaches. One approach is based on an evaluation of the phosphorylation state, either unphosphorylated (inactive) or phosphorylated (active). The phosphorylated protein

has a slower mobility in SDS-PAGE and can therefore be compared with the unstimulated protein using Western blotting. Alternatively, antibodies specific for the phosphorylated protein are available (New England Biolabs) which can be used to detect an increase in the phosphorylated kinase. In either method, cells are stimulated with the mitogen and then extracted with Laemmli buffer. The soluble fraction is applied to an SDS-PAGE gel and proteins are transferred electrophoretically to nitrocellulose or Immobilon. Immunoreactive bands are detected by standard Western blotting technique. Visible or chemiluminescent signals are recorded on film and may be quantified by densitometry.

Another approach is based on evaluation of the MAP kinase activity via a phosphorylation assay. Cells are stimulated with the mitogen and a soluble extract is prepared. The extract is incubated at 30°C for 10 min with gamma-32-ATP, an ATP regenerating system, and a specific substrate for MAP kinase such as phosphorylated heat and acid stable protein regulated by insulin, or PHAS-I. The reaction is terminated by the addition of H₃PO₄ and samples are transferred to ice. An aliquot is spotted onto Whatman P81 chromatography paper, which retains the phosphorylated protein. The chromatography paper is washed and counted for ³²P in a liquid scintillation counter. Alternatively, the cell extract is incubated with gamma-32-ATP, an ATP regenerating system, and biotinylated myelin basic protein bound by streptavidin to a filter support. The myelin basic protein is a substrate for activated MAP kinase. The phosphorylation reaction is carried out for 10 min at 30°C. The extract can then be aspirated through the filter, which retains the phosphorylated myelin basic protein. The filter is washed

and counted for ^{32}P by liquid scintillation counting.

Cell proliferation assay

Activation of a G protein coupled receptor may lead to a mitogenic or proliferative response which can be monitored via $[^3\text{H}]\text{-thymidine}$ uptake. When cultured cells are incubated with $[^3\text{H}]\text{-thymidine}$, the thymidine translocates into the nuclei where it is phosphorylated to thymidine triphosphate. The nucleotide triphosphate is then incorporated into the cellular DNA at a rate that is proportional to the rate of cell growth. Typically, cells are grown in culture for 1-3 days. Cells are forced into quiescence by the removal of serum for 24 hrs. A mitogenic agent is then added to the media. 24 hrs later, the cells are incubated with $[^3\text{H}]\text{-thymidine}$ at specific activities ranging from 1 to 10 $\mu\text{Ci}/\text{ml}$ for 2-6 hrs. Harvesting procedures may involve trypsinization and trapping of cells by filtration over GF/C filters with or without a prior incubation in TCA to extract soluble thymidine. The filters are processed with scintillant and counted for ^3H by liquid scintillation counting. Alternatively, adherent cells are fixed in MeOH or TCA, washed in water, and solubilized in 0.05% deoxycholate/0.1 N NaOH. The soluble extract is transferred to scintillation vials and counted for ^3H by liquid scintillation counting.

Methods for recording currents in *Xenopus* oocytes

Female *Xenopus laevis* (*Xenopus*-1, Ann Arbor, MI) are anesthetized in 0.2% tricain (3-aminobenzoic acid ethyl ester, Sigma Chemical Corp.) and a portion of ovary is removed using aseptic technique (Quick and Lester, 1994). Oocytes are defolliculated using 2 mg/ml collagenase (Worthington Biochemical Corp., Freehold, NJ) in a

solution containing 87.5 mM NaCl, 2 mM KCl, 2 mM MgCl₂ and 5 mM HEPES, pH 7.5. Oocytes may be injected (Nanoject, Drummond Scientific, Broomall, PA) with mammalian mRNA. Other oocytes may be injected with a mixture of mammalian mRNA and mRNA encoding the genes for G-protein-activated inward rectifiers (GIRK1 and GIRK4, U.S. Patent Nos. 5,734,021 and 5,728,535). Genes encoding G-protein inwardly rectifying K⁺ (GIRK) channels 1 and 4 (GIRK1 and GIRK4) were obtained by PCR using the published sequences (Kubo et al., 1993; Dascal et al., 1993; Krapivinsky et al., 1995 and 1995b) to derive appropriate 5' and 3' primers. Human heart cDNA was used as template together with the primers
5 5'-CGCGGATCCATTATGTCTGCACTCCGAAGGAAATTTG-3' (SEQ ID NO:
10 10) and
15 5'-CGCGAATTCTTATGTGAAGCGATCAGAGTTCATTTTC-3' (SEQ ID NO:
11) for GIRK1 and
5'-GCGGGATCCGCTATGGCTGGTGATTCTAGGAATG-3' (SEQ ID NO: 12)
and
20 5'- CCGGAATTCCCCTCACACCGAGGCCCTGG-3' (SEQ ID NO: 13) for
GIRK4.
In each primer pair, the upstream primer contained a BamHI site and the downstream primer contained an EcoRI site to facilitate cloning of the PCR product into pcDNA1-Amp
25 (Invitrogen). The transcription template for the mammalian receptor may be similarly obtained. mRNAs are prepared from separate DNA plasmids containing the complete coding regions of the mammalian receptor, GIRK1, and GIRK4. Plasmids are linearized and transcribed using
30 the T7 polymerase ("Message Machine", Ambion). Alternatively, mRNA may be translated from a template generated by PCR, incorporating a T7 promoter and a poly A⁺ tail. Each oocyte receives 2 ng each of GIRK1 and GIRK4 mRNA in combination with 25 ng of mammalian receptor mRNA.
35 After injection of mRNA, oocytes are incubated at 16° C on

a rotating platform for 3-8 days. Dual electrode voltage clamp ("GeneClamp", Axon Instruments Inc., Foster City, CA) is performed using 3 M KCl-filled glass microelectrodes having resistances of 1-3 Mohms. Unless otherwise specified, oocytes are voltage clamped at a holding potential of -80 mV. During recordings, oocytes are bathed in continuously flowing (2-5 ml/min) medium containing 96 mM NaCl, 2 mM KCl, 2 mM CaCl₂, 2 mM MgCl₂, and 5 mM HEPES, pH 7.5 ("ND96"), or, in the case of oocytes expressing GIRK1 and GIRK4, elevated K⁺ containing 96 mM KCl, 2 mM NaCl, 2 mM CaCl₂, 2 mM MgCl₂, and 5 mM HEPES, pH 7.5 ("hK"). Drugs are applied by switching from a series of gravity fed perfusion lines.

Heterologous expression of GPCRs in *Xenopus* oocytes has been widely used to determine the identity of signaling pathways activated by agonist stimulation (Gundersen et al., 1983; Takahashi et al., 1987). Activation of the phospholipase C (PLC) pathway is assayed by applying test compound in ND96 solution to oocytes previously injected with mRNA for the mammalian receptor and observing inward currents at a holding potential of -80 mV. The appearance of currents that reverse at -25 mV and display other properties of the Ca⁺⁺-activated Cl⁻ (chloride) channel is indicative of mammalian receptor-activation of PLC and release of IP₃ and intracellular Ca⁺⁺. Such activity is exhibited by GPCRs that couple to G_q.

Measurement of inwardly rectifying K⁺ (potassium) channel (GIRK) activity is monitored in oocytes that have been co-injected with mRNAs encoding the mammalian receptor, GIRK1, and GIRK4. The two GIRK gene products co-assemble to form a G-protein activated potassium channel known to be activated (i.e., stimulated) by a number of GPCRs that couple to G_i or G_o (Kubo et al., 1993; Dascal et al.,

1993). Oocytes expressing the mammalian receptor plus the
two GIRK subunits are tested for test compound
responsivity by measuring K⁺ currents in elevated K⁺
solution (hK). Activation of inwardly rectifying currents
5 that are sensitive to 300 μM Ba⁺⁺ signifies the mammalian
receptor coupling to a G_i or G_o pathway in the oocytes.

Receptor/G protein co-transfection studies

A strategy for determining whether MCH1 can couple
10 preferentially to selected G proteins involves co-
transfection of MCH1 receptor cDNA into a host cell
together with the cDNA for a G protein alpha sub-unit.
Examples of G alpha sub-units include members of the
15 G_{αi}/G_{αo} class (including G_{αt2} and G_{αz}), the G_{αq} class, the
G_{αs} class, and the G_{α12/13} class. A typical procedure
involves transient transfection into a host cell such as
20 COS-7. Other host cells may be used. A key consideration
is whether the cell has a downstream effector (a
particular adenylate cyclase, phospholipase C, or channel
isoform, for example) to support a functional response
through the G protein under investigation. G protein beta
gamma sub-units native to the cell are presumed to
complete the G protein heterotrimer; otherwise specific
25 beta and gamma sub-units may be co-transfected as well.
Additionally, any individual or combination of alpha,
beta, or gamma subunits may be co-transfected to optimize
the functional signal mediated by the receptor.

The receptor/G alpha co-transfected cells are evaluated in
30 a binding assay, in which case the radioligand binding may
be enhanced by the presence of the optimal G protein
coupling or in a functional assay designed to test the
receptor/G protein hypothesis. In one example, the MCH1
receptor may be hypothesized to inhibit cAMP accumulation
35 through coupling with G alpha sub-units of the G_{αi}/G_{αo}

class. Host cells co-transfected with the MCH1 receptor and appropriate G alpha sub-unit cDNA are stimulated with forskolin +/- MCH1 agonist, as described above in cAMP methods. Intracellular cAMP is extracted for analysis by radioimmunoassay. Other assays may be substituted for cAMP inhibition, including GTP γ ³⁵S binding assays and inositol phosphate hydrolysis assays. Host cells transfected with MCH1 minus G alpha or with G alpha minus MCH1 would be tested simultaneously as negative controls.

10 MCH1 receptor expression in transfected cells may be confirmed in radioligand binding studies using membranes from transfected cells. G alpha expression in transfected cells may be confirmed by Western blot analysis of membranes from transfected cells, using antibodies specific for the G protein of interest.

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The efficiency of the transient transfection procedure is a critical factor for signal to noise in an inhibitory assay, much more so than in a stimulatory assay. If a positive signal present in all cells (such as forskolin-stimulated cAMP accumulation) is inhibited only in the fraction of cells successfully transfected with receptor and G alpha, the signal to noise ratio will be poor. One method for improving the signal to noise ratio is to create a stably transfected cell line in which 100% of the cells express both the receptor and the G alpha subunit. Another method involves transient co-transfection with a third cDNA for a G protein-coupled receptor which positively regulates the signal which is to be inhibited.

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If the co-transfected cells simultaneously express the stimulatory receptor, the inhibitory receptor, and a requisite G protein for the inhibitory receptor, then a positive signal may be elevated selectively in transfected cells using a receptor-specific agonist. An example involves co-transfection of COS-7 cells with 5-HT4

receptor, MCH1 receptor, and a G alpha sub-unit. Transfected cells are stimulated with a 5-HT4 agonist +/- MCH1 agonist. Cyclic AMP is expected to be elevated only in the cells also expressing MCH1 and the G alpha subunit of interest, and a MCH1-dependent inhibition may be measured with an improved signal to noise ratio.

It is to be understood that the cell lines described herein are merely illustrative of the methods used to evaluate the binding and function of the mammalian receptors of the present invention, and that other suitable cells may be used in the assays described herein.

15 Promiscuous second messenger assays

It is possible to coax receptors of different functional classes to signal through a pre-selected pathway through the use of promiscuous G_{α} subunits. For example, by providing a cell based receptor assay system with an exogenously supplied promiscuous G_{α} subunit such as $G_{\alpha 16}$ or a chimeric G_{α} subunit such as $G_{\alpha qz}$, a GPCR which normally might prefer to couple through a specific signaling pathway (e.g. G_s , G_i , G_q , G_o , etc.), can be made to couple through the pathway defined by the promiscuous G_{α} subunit and upon agonist activation produce the second messenger associated with that subunit's pathway. In the case of $G_{\alpha 16}$ and/or $G_{\alpha qz}$ this would involve activation of the G_q pathway and production of the second messenger inositol phosphate. Through similar strategies and tools, it is possible to bias receptor signaling through pathways producing other second messengers such as Ca^{++} , cAMP, K^+ currents, etc.

35 Microphysiometric assay

Because cellular metabolism is intricately involved in and effected by a broad range of cellular events (including

receptor activation of various second messenger pathways),
the use of microphysiometric measurements of cell
metabolism can in principle provide a generic assay of
cellular activity arising from the activation of any
5 receptor regardless of the specifics of the receptor's
proximal signaling pathway.

General guidelines for cell preparation and
microphysiometric recording have been previously reported
10 (Salon, J.A. and Owicki, J.A., 1996). A typical protocol
employing transiently transfected CHO cells is as follows;
24 hours prior to recording, transfected cells are
harvested and counted. 3×10^5 cells are seeded into cell
culture capsules (Costar), and allowed to attach to the
15 capsule membrane. 10 hours later (14 hours prior to
recording) the cell media is switched to serum free F-12
complete to minimize ill-defined metabolic stimulation
caused by assorted serum factors.

20 On the day of the experiment the cell capsules are
transferred to the microphysiometer (Cytosensor, Molecular
Devices Corporation, Sunnyvale, CA) and allowed to
equilibrate in recording media (low buffered RPMI 1640, no
bicarbonate, no serum) with 0.1% BSA (essentially fatty
25 acid free), during which a baseline measurement of basal
metabolic activity is established. The recording paradigm
consists of a 100 μ l/min flow rate, with a 2 min pump
cycle which includes a 30 sec flow interruption during
which the rate measurement is taken. Challenges involve
30 a 1 min 20 sec exposure to a drug just prior to the first
post challenge rate measurement being taken, followed by
two additional pump cycles for a total of 5 min 20 sec
drug exposure. Drug is then washed out and rates allowed
35 to return to basal. Reported extracellular acidification
rates are expressed as a percentage increase of the peak

response over the baseline rate observed just prior to challenge.

GPCR ligand library

5 Functional assays of new receptors such as MCH1 may include a preliminary test of a small library of compounds containing representative agonists for all known GPCRs as well as other compounds which may be agonists for prospective GPCRs or which may be effectors for targets peripherally involved with GPCRs. The collection used in
10 this study comprises approximately 180 compounds (including small molecules, hormones, preprohormones, peptides, etc.) for more than 45 described classes of GPCRs (serotonin, dopamine, noradrenaline, opioids, etc.)
15 and additionally includes ligands for known or suspected but not necessarily pharmacological characterized or cloned GPCR families (such as MCH).

20 The diversity of the library can be expanded to include agonist and antagonist compounds specific for GPCR subtypes, combinatorial peptide and/or small molecule libraries, natural product collections, and the like. To facilitate robotic handling, the substances are distributed as either separate or pooled compound concentrates in 96 well plates and stored frozen as ready
25 to use reagent plates.

Localization of mRNA coding for human MCH1 receptors

30 **Development of probes for MCH1:** To facilitate the production of radiolabeled, antisense RNA probes a fragment of the gene encoding rat MCH1 will be subcloned into a plasmid vector containing RNA polymerase promoter sites. The full length cDNA encoding the rat MCH1 will be digested with Pst I, (nucleotides 905-1194) and this 289 nucleotide fragment will be cloned into the Pst I site of
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5 pGEM 3z, containing both sp6 and T7 RNA polymerase promoter sites. The construct will be sequenced to confirm sequence identity and orientation. To synthesize antisense strands of RNA, this construct will be linearized with Hind III or Eco RI (depending on orientation) and T7 or sp6 RNA polymerase will be used to incorporate radiolabeled nucleotide as described below.

10 A probe coding for the rat glyceraldehyde 3-phosphate dehydrogenase (GAPDH) gene, a constitutively expressed protein, was used concurrently. GAPDH is expressed at a relatively constant level in most tissue and its detection is used to compare expression levels of the rat MCH1 receptors gene in different regions.

15 **Synthesis of probes:** MCH1 and GAPDH cDNA sequences preceded by phage polymerase promoter sequences will be used to synthesize radiolabeled riboprobes. Conditions for the synthesis of riboprobes will be: 0.25-1.0 μ g linearized DNA plasmid template, 1.5 μ l of ATP, GTP, UTP (10 mM each), 3 μ l dithiothreitol (0.1 M), 30 units RNasin RNase inhibitor, 0.5-1.0 μ l (15-20 units/ μ l) RNA polymerase, 7.0 μ l transcription buffer (Promega Corp.), and 12.5 μ l α^{32} P-CTP (specific activity 3,000Ci/mmol). 0.1 mM CTP (0.02-1.0 μ l) will be added to the reactions, and the volume will be adjusted to 35 μ l with DEPC-treated water. Labeling reactions will be incubated at 37°C for 60 min, after which 3 units of RQ1 RNase-free DNase (Promega Corp.) will be added to digest the template.

20 Riboprobes will be separated from unincorporated nucleotides using Microspin S-300 columns (Pharmacia Biotech). TCA precipitation and liquid scintillation spectrometry will be used to measure the amount of label incorporated into the probe. A fraction of all riboprobes synthesized will be size-fractionated on 0.25 mm thick 7M

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urea, 4.5% acrylamide sequencing gels. These gels will be apposed to storage phosphor screens and the resulting autoradiograph scanned using a phosphorimager (Molecular Dynamics, Sunnyvale, CA) to confirm that the probes synthesized were full-length and not degraded.

Solution hybridization/ribonuclease protection assay (RPA): For solution hybridization 2.0 μ g of mRNA isolated from tissues will be used. Negative controls consisted of 30 μ g transfer RNA (tRNA) or no tissue blanks. All mRNA samples will be placed in 1.5-ml microfuge tubes and vacuum dried. Hybridization buffer (40 μ l of 400 mM NaCl, 20 mM Tris, pH 6.4, 2 mM EDTA, in 80% formamide) containing 0.25-2.0 E⁶ counts of each probe will be added to each tube. Samples will be heated at 95°C for 15 min, after which the temperature will be lowered to 55°C for hybridization.

After hybridization for 14-18 hr, the RNA/probe mixtures will be digested with RNase A (Sigma) and RNase T1 (Life Technologies). A mixture of 2.0 μ g RNase A and 1000 units of RNase T1 in a buffer containing 330 mM NaCl, 10 mM Tris (pH 8.0) and 5 mM EDTA (400 μ l) will be added to each sample and incubated for 90 min at room temperature. After digestion with RNases, 20 μ l of 10% SDS and 50 μ g proteinase K will be added to each tube and incubated at 37°C for 15 min. Samples will be extracted with phenol/chloroform:isoamyl alcohol and precipitated in 2 volumes of ethanol for 1 hr at -70°C. Pellet Paint (Novagen) will be added to each tube (2.0 μ g) as a carrier to facilitate precipitation. Following precipitation, samples will be centrifuged, washed with cold 70% ethanol, and vacuum dried. Samples will be dissolved in formamide loading buffer and size-fractionated on a urea/acrylamide sequencing gel (7.0 M urea, 4.5% acrylamide in Tris-

borate-EDTA). Gels will be dried and apposed to storage phosphor screens and scanned using a phosphorimager (Molecular Dynamics, Sunnyvale, CA).

5 **RT-PCR:** For the detection of RNA encoding human MCH1, RT-PCR was carried out on mRNA extracted from human tissue. Reverse transcription and PCR reactions were carried out in 50 ml volumes using EZrTth DNA polymerase (Perkin Elmer). Primers with the following sequences were used:

10 *Forward primer (RA SLC1a /MCH F); TCA GCT CGG TTG TGG*
 GAG CA (SEQ ID NO: 14)
 Reverse primer (RA/SLC1a MCH B); CTT GGA CTT CTT CAC
 GAC (SEQ ID NO: 15)

15 These primers will amplify a 248 base pair fragment from nucleotide 169 to 417.

20 Each reaction contained 0.1 μ g mRNA and 0.3 μ M of each primer. Concentrations of reagents in each reaction were: 300 μ M each of GTP; dATP; dCTP; dTTP; 2.5mM Mn(OAc)₂; 50 mM Bicine; 115 mM potassium acetate, 8% glycerol and 5 units EZrTth DNA polymerase. All reagents for PCR (except mRNA and oligonucleotide primers) were obtained from Perkin Elmer. Reactions were carried out under the following conditions: 65°C 60 min., 94°C 2 min., (94°C, 1 min., 65°C 1 min) 35 cycles, 72°C 10 min. PCR reactions were size fractionated by gel electrophoresis using 10% polyacrylamide. DNA was stained with SYBR Green I (Molecular Probes, Eugene OR) and scanned on a Molecular Dynamics (Sunnyvale CA) Storm 860 in blue fluorescence mode at 450 nM.

Positive controls for PCR reactions consisted of

amplification of the target sequence from a plasmid construct, as well as reverse transcribing and amplifying a known sequence. Negative controls consisted of mRNA blanks, as well as primer and mRNA blanks. To confirm
5 that the mRNA was not contaminated with genomic DNA, samples were digested with RNases before reverse transcription. Integrity of RNA was assessed by amplification of mRNA coding for GAPDH.

Receptor Audioradiographic Experiments Localizing the MCH1 Receptor in the rat CNS

Animals

5 Male Sprague-Dawley rats (Charles Rivers, Rochester, NY) were euthanized using CO₂ and decapitated and their brains rapidly removed and frozen on crushed dry ice. Coronal sections were cut at 20μm using a cryostat and thaw-mounted onto gelatin-coated slides then stored at -20°C
10 until use.

Radioligand Binding Studies

15 In radioligand binding assays [³H]Compound 10 (specific activity 56 Ci/mmol (NEN, Boston, MA) was used at 0.1 nM. Dopamine, prazosin, and phenanthroline were obtained from Sigma (St. Louis, MO). Phenylmethylsulfonyl Fluoride (PMSF) was from Calbiochem (La Jolla, CA).

In vitro autoradiography

20 Tissue sections were allowed to equilibrate to room temperature for one hour. Sections were incubated at 25°C for 1.5 hours in 50 mM Tris-HCl buffer, pH 7.4, containing 10 mM MgCl₂, 0.16 mM PMSF, 0.3 mM phenanthroline, 0.2% bovine serum albumin (Boehringer Mannheim, Indianapolis, IN), 100 μM dopamine, 1 μM prazosin, and 0.01 nM [³H]Compound 10. Nonspecific binding was determined by including 10 μM unlabeled Compound 10 in the incubation buffer. Following incubation the sections were washed twice for 5 minutes each in 4°C 50 mM Tris-buffer, pH 7.4, then rapidly dipped in ice-cold distilled water to remove the salts. Tissues were dried under a stream of cold air and apposed together with ³H-plastic standard scales, to Hyperfilm-³H (Amersham, Piscataway, NJ) for 6 weeks. Films were developed using a Kodak developer-D19 and Rapid fixer (Kodak, Rochester, NY). Specific [³H]Compound 10 binding
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to the MCH1 receptor was interpreted by observation of the remaining optical density on the autoradiogram in the various regions of rat brain in the presence of the appropriate displacers.

Chemical Synthetic Methods

5 **General Methods:** All reactions (except for those done by parallel synthesis reaction arrays) were performed under an Argon atmosphere and the reagents, neat or in appropriate solvents, were transferred to the reaction vessel via syringe and cannula techniques. The parallel
10 synthesis reaction arrays were performed in vials (without an inert atmosphere) using J-KEM heating shakers (Saint Louis, MO). Anhydrous solvents were purchased from Aldrich Chemical Company and used as received. The examples described in the patent (1-37) were named using
15 ACD/Name program (version 2.51, Advanced Chemistry Development Inc., Toronto, Ontario, M5H2L3, Canada). Unless otherwise noted, the ^1H and ^{13}C NMR spectra were recorded at 300 and 75 MHz (QE Plus) with CDCl_3 , as solvent and tetramethylsilane as internal standard. s = singlet;
20 d = doublet; t = triplet; q = quartet; p = pentet; sextet; septet; br = broad; m = multiplet. Elemental analyses were performed by Robertson Microlit Laboratories, Inc. Unless otherwise noted, mass spectra were obtained using low-resolution electrospray (ESMS) and MH^+ is reported.
25 Thin-layer chromatography (TLC) was carried out on glass plates precoated with silica gel 60 F254 (0.25 mm, EM Separations Tech.). Preparative thin-layer chromatography was carried out on glass sheets precoated with silica gel GF (2 mm, Analtech). Flash column chromatography was
30 performed on Merck silica gel 60 (230 - 400 mesh). Melting points (mp) were determined in open capillary tubes on a Mel-Temp apparatus and are uncorrected.

Procedures for the Synthesis of the Dihydropyrimidine Intermediates

5 5-METHOXCARBONYL-4-METHOXYMETHYL-1,2,3,6-TETRAHYDRO-2-OXO-6-(3,4-DIFLUOROPHENYL)-PYRIMIDINE: To a stirring mixture of methyl 4-methoxyacetacetate (50.0 g, 0.342 mol), 3,4-difluorobenz-aldehyde (51.4 g, 0.362 mol), and urea (31.6 g, 0.527 mole) in THF (300 mL) at room temperature were added copper(I) oxide (5.06 g, 0.035 mole) and acetic acid (2.05 mL), sequentially, followed by dropwise addition of boron trifluoride diethyl etherate (56.0 mL, 0.442 mole). The mixture was stirred and refluxed for 8 h, whereupon TLC (1/1 EtOAc/hexanes) analysis indicated completion of the reaction. The reaction mixture was cooled and poured into a mixture of ice and sodium bicarbonate (100 g) and the resulting mixture was filtered through Celite. The Celite pad was washed with dichloromethane (400 mL). The organic layer was separated from the filtrate and the aqueous layer was extracted with more dichloromethane (3 X 300 mL). The combined organic extracts were dried (sodium sulfate) and the solvent evaporated. The crude product was purified by flash column (ethyl acetate/hexanes, 1/1; then ethyl acetate), giving the product as pale yellow foam, which on trituration with hexane became white powder (103 g, 97%).
1H NMR d 3.48 (s, 3H), 3.65 (s, 3H), 4.65 (s, 2H), 5.39 (s, 1H), 6.60 (br s, 1H, NH), 7.00 - 7.20 (m, 3H), 7.72 (br s, 1H, NH).

30 (+)-5-METHOXCARBONYL-4-METHOXYMETHYL-1,2,3,6-TETRAHYDRO-2-OXO-6-(3,4-DIFLUOROPHENYL)-PYRIMIDINE: The racemic intermediate 5-methoxycarbonyl-4-methoxymethyl-1,2,3,6-tetrahydro-2-oxo-6-(3,4-difluorophenyl)pyrimidine was resolved by chiral HPLC [Chiralcel OD 20 X 250 mm #369-703-30604;

lambda 254 nm; hexanes/ethanol 90/10; 85 mg per injection; retention time of the desired enantiomer: 16.94 min., the first enantiomer peak to elute], giving (+)-5-methoxycarbonyl-4-methoxymethyl-1,2,3,6-tetrahydro-2-oxo-6-(3,4-difluorophenyl)-pyrimidine (40-42 wt% isolation of the desired enantiomer from the racemate); $[\alpha]_D = + 83.8$ ($c = 0.5$, chloroform). The (-)-isomer was also isolated as the later eluting fraction from the chiral chromatography column.

(+) - 5 - METHOXYCARBONYL - 4 - METHOXYMETHYL - 1 , 2 , 3 , 6 - TETRAHYDRO
- 2 - O X O -
6 - (3 , 4 - DIFLUOROPHENYL) - 1 - [(4 - NITROPHENYLOXY) CARBONYL] PYR
I M I D I N E : To a s o l u t i o n o f
(+) - 5 - methoxycarbonyl - 4 - methoxymethyl - 1 , 2 , 3 , 6 -
tetrahydro - 2 - oxo - 6 - (3 , 4 - difluorophenyl) - pyrimidine (1 . 9 8
g, 6.34 mmol) in anhydrous THF (2 0 mL) at - 7 8 ° C under
argon atmosphere, a solution of lithium
hexamethyldisilazide in THF (1 M, 18.0 mL, 18.0 mmol) was
added over 2-3 min. and the mixture was stirred for 10
min. This solution was added over 6 min., via a cannula,
to a stirred solution of 4-nitrophenyl chloroformate (4 . 4 7
g, 22.2 mmol) in THF (2 0 mL) at - 7 8 ° C. Stirring was
continued for 10 min. and the mixture was poured onto ice
(5 0 g) and extracted with chloroform (2 X 5 0 mL). The
combined extracts were dried (sodium sulfate) and the
solvent was evaporated. The residue was purified by flash
column chromatography (hexanes/ethyl acetate, 4/1 to
3.5/1) as the eluent. The product was obtained as yellow
syrup which upon trituration with hexanes became a white
powder (2 . 4 0 g, 79%): ^1H NMR d 3.52 (s, 3H), 3.74 (s, 3H),
4.65-4.80 (q, J=16.5 Hz, 2H), 6.32 (s, 1H), 7.10-7.30 (m,
4H), 7.36 (d, J=9 Hz, 2H), 8.27 (d, J=9 Hz, 2H).

BENZYL 3 - [(3 , 4 - DIFLUOROPHENYL) METHYLENE] - 4 - OXOPENTANOATE :

A solution of benzyl propionylacetate (36.3 g, 176 mmol), 3,4- difluorobenzaldehyde (25.0 g, 176 mmol), piperidine (0.86 mL, 9.0 mmol) and acetic acid (0.49 mL, 9.0 mmol) was refluxed with removal of water using a Dean-Stark apparatus for 5 h. The solvent was removed *in vacuo* and the residue was dissolved in EtOAc. The reaction mixture was washed with water (100 mL), followed by brine (100 mL) and dried over anhydrous Na₂SO₄. The solvent was evaporated, giving a pale yellow syrup (60.2 g). The product was used in the next step without further purification.

5 - (BENZYLOXYCARBONYL) -1,6-DIHYDRO-2-METHOXY-4-ETHYL-6-(3 ,4-DI-FLUOROPHENYL) PYRIMIDINE: A suspension of benzyl 3-[(3,4-di-fluorophenyl)methylene]-4-oxopentanoate (16.0 g, 48.0 mmol), O-methylisourea hydrogen sulfate (16.7 g, 97.0 mmol) and NaHCO₃ (16.3 g, 130 mmol) in DMF (190 mL) was stirred at 70 °C for 20 h. After cooling to room temperature, the mixture was filtered and the filtrate was diluted with EtOAc (300 mL) and then washed with water (4X100 mL), brine (200 mL) and dried over Na₂SO₄. After removal of solvent, the residue was purified by column chromatography (EtOAc/Hexane, 1/9 to 3/7), giving the title compound as a colorless oil (10.6 g, 58%). The NMR analysis showed it to be a mixture of amine/imine tautomers and was used as is in the next step.

5 - (BENZYLOXYCARBONYL) -4-ETHYL-1,6-DIHYDRO-2-METHOXY-6-(3 ,4-DI-FLUOROPHENYL) -1- [(4-NITROPHENYLOXY) CARBONYL] PYRIMIDINE: To a stirring solution of 5 - (benzyl oxy carbonyl) -1,6-dihydro -2 - methoxy-4-ethyl-6-(3,4-difluorophenyl)pyrimidine (17.0 g, 44.0 mmol) and 4-dimethylaminopyridine (7.00 g, 57.3 mmol) in CH₂Cl₂ (200 mL) was added 4-nitrophenyl chloroformate as

a powder (11.5 g, 57.1 mmol) at room temperature. The reaction mixture was stirred for 12 h and then the solvent was removed *in vacuo*. The residue was purified by chromatography (EtOAc/Hexane, 1/9 to 3/7), giving
5 5-(benzyloxycarbonyl)-4-ethyl-1,6-dihydro-2-methoxy-6-(3,4-difluorophenyl)-1-[(4-nitrophenyloxy)carbonyl]pyr-imidine as a colorless viscous oil (12.6 g, 50%).
10 ^1H NMR δ 1.24 (t, J=7.2 Hz, 3H), 2.81-2.98 (m, 3H), 3.97 (s, 3H), 5.14 (ABq, A=5.08, B= 5.20, J= 12.3 Hz, 2H),
6.28 (s, 3H), 7.03-7.29 (m, 8H), 7.35 (d, J=9.2 Hz, 2H),
8.26 (d, J=9.2 Hz, 2H).

5-(BENZYLOXYCARBONYL)-4-ETHYL-1,6-DIHYDRO-1-{N-[1-PHENYL
]) E T H Y L] } -
15 CARBOXAMIDO-2-METHOXY-6-(3,4-DIFLUOROPHENYL) PYRIMIDINE:
To a stirred mixture of 5-(benzyloxycarbonyl)-4-ethyl-1,6-dihydro-2-methoxy-6-(3,4-difluorophenyl)-1-[(4-nitrophenyloxy)carbonyl]pyr-imidine (12.6 g, 22.9 mmol) in THF (150 mL) was added a solution of R-(+)-α-methyl benzylamine (3.53 mL, 27.1 mmol) at room temperature. The stirring was continued for 12 h and the solvent was removed *in vacuo*. The yellow residue was dissolved in chloroform (200 mL) and was washed with 10% K_2CO_3 solution (2x30 mL). The organic layer was dried over Na_2SO_4 , filtered and solvent was removed *in vacuo*. The resulting mixture of diastereomers was separated by column chromatography (petroleum ether/ether, 9/1 to 4/1). The first major product to elute was (+)-5-(benzyloxycarbonyl)-4-ethyl-1,6-dihydro-1-{N-[1-
20 p h e n y l] } carboxamido-2-methoxy-6-(3,4-difluorophenyl)pyrimidine. Colorless oil; R_f = 0.31 (petroleum ether/ether, 4/1); yield: 3.8 g (31%); $[\alpha]_D = +267.05$ ($c = 0.76, \text{CHCl}_3$);
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1H NMR δ 1.22 (t, J=7.5 Hz, 3H), 1.52 (d, J=6.9 Hz, 3H),
2.88 (q, J=6.0 Hz, 2H), 3.99 (s, 3H), 4.99 (m, 1H), 5.09
(ABq, A=5.00, B= 5.19, J= 12.6 Hz, 2H), 6.66 (s, 1H),
6.99-7.36 (m, 13H). The second major product to elute was
5 (-)-5-(benzyloxycarbonyl)-4-ethyl-1,6-dihydro-1-{N-[2-phenyl]ethyl}carboxamido-2-methoxy-6-(3,4-difluorophenyl)pyr-imidine. Colorless oil; Rf= 0.22 (petroleum ether/ether, 4/1); yield: 3.20 g (26%); [α]_D = -146.89 (c = 0.38, CHCl₃); ¹H NMR δ 1.22 (t, J=7.2 Hz, 3H), 1.49 (d, J=6.6 Hz, 3H), 2.88 (q, J=6.0 Hz, 2H), 3.94 (s, 3H),
10 5.03 (m, 1H), 5.11 (ABq, A=5.02, B= 5.19, J= 12.6 Hz, 2H), 6.68 (s, 1H), 6.91-7.34 (m, 13H).

15 (+)-5-(BENZYLOXYCARBONYL)-1,6-DIHYDRO-2-METHOXY-4-ETHYL-
6-(3,4-DI-FLUOROPHENYL) PYRIMIDINE: To a stirred solution
o f (+) - 5 - (b e n z - yloxy carbonyl) - 4 - ethyl - 1 , 6 - dihydro - 1 - { N - [2 - phenyl] ethyl } carbox - amido - 2 - methoxy - 6 - (3 , 4 - difluorophenyl) pyrimidine (1.00 g, 1.83 mmol) in toluene (10 mL) was added
20 1,8-diazabicyclo[5.4.0]-undec- 7-ene (0.120 mL, 0.810 mmol) at room temperature and the resulting solution was heated at reflux temperature for 5 h and then stirred for 12 h at room temperature. The solvent was evaporated and the residue was purified by flash column (EtOAc/Hexanes, 1/3), giving (+)-5-(benzyloxycarbonyl)-1,6-dihydro-2-methoxy-4-ethyl-6-(3,4-difluorophenyl)pyrimidine (0.560 g, 77%).

30 (+)-5-(BENZYLOXYCARBONYL)-4-ETHYL-1,6-DIHYDRO-2-METHOXY-
6 - (3 , 4 - , 4 - - D I - -
FLUOROPHENYL) - 1 - [(4 - NITROPHENYLOXY) CARBONYL] PYRIMIDINE:
T o a s t i r r i n g s o l u t i o n o f
(+) - 5 - (b e n z y l o x y c a r b o n y l) - 1 , 6 - d i h y d r o - 2 -
methoxy-4-ethyl-6-(3,4-difluorophenyl)pyrimidine (17.0 g,

44.0 mmol) and 4-dimethylaminopyridine (6.99 g, 57.3 mmol) in CH_2Cl_2 (200 mL) was added 4-nitrophenyl chloroformate (11.6 g, 57.3 mmol) at room temperature. The reaction mixture was stirred for 12 h and then the solvent was removed *in vacuo*. The residue was purified by chromatography (EtOAc/Hexane, 1/9 to 3/7), giving (+)-5-(benzyloxycarbonyl)-4-ethyl-1,6-dihydro-2-methoxy-6-(3,4-difluorophenyl)-1-[(4-nitrophenyloxy)carbonyl]pyrimidine as a viscous colorless oil (19.3 g, 76%).

5-METHYLBENZFUROXAN: 4-Methyl-2-nitroaniline (100 g, 0.650 mol) was suspended in saturated methanolic sodium hydroxide solution (1.50 L). This suspension was cooled (5 °C) and aqueous sodium hypochlorite until the red color disappeared. The resulting fluffy yellow precipitate was filtered, washed with cold water and recrystallized from ethanol, giving 5-methylbenzfuroxan (88.2 g, 89 % yield) as a pale yellow solid: ^1H NMR δ 2.39 (s, 3 H), 6.90-7.40 (br m, 3 H).

5-METHYLBENZOFURAZAN: To 5-Methylbenzfuroxan (88.2 g, 0.590 mol) in refluxing EtOH (75 mL) was added dropwise $\text{P}(\text{OEt})_3$ (150 mL). Heating was continued at reflux temperature for 1 h. The solvent was removed *in vacuo* and the residue was shaken with water (200 mL) and allowed to stand overnight at (0-5 °C). The resulting brown solid was filtered, washed with water. The crude product was purified by flash chromatography, giving 5-methylbenzofurazan (70.0 g, 87 %) as white needles; ^1H NMR δ 2.41 (s, 1 H), 7.19 (dd, $J=9.3, 1.1$ Hz, 1 H), 7.48 (d, $J=1.1$ Hz, 1 H), 7.66 (d, $J=9.3$ Hz, 1 H).

5-DIBROMOMETHYLBENZOFURAZAN: An anhydrous solution of 5-methylbenzofurazan (70.0 g, 0.520 mol),

N-bromosuccinamide (325 g), and benzoyl peroxide (0.50 g) in carbon tetrachloride (1.5 L) was heated at reflux temperature with stirring for 30 h. The reaction mixture was washed with water (2 X 500 mL), dried (NaSO_4), and the solvent was removed *in vacuo*. The residue was chromatographed (EtOAc/hexane, 1/150), giving 122 g (80%) of the title compound as a white solid: ^1H NMR δ 6.69 (s, 1 H), 7.69 (d, $J=9.6$ Hz, 1 H), 7.77 (s, 1 H), 7.89 (d, $J=9.6$ Hz, 1 H).

5-FORMYL BENZOFURAZAN: AgNO_3 (163 g) in 2 L of water was added to a refluxing mixture of dibromomethylbenzofurazan (122 g, 418 mmol) in EtOH (1 L). Heating at reflux temperature was continued for 2 h. The mixture was cooled, the precipitated AgBr was removed by filtration through Celite, and the solvent was concentrated. The resulting solution was extracted with toluene (10 X 100 mL), dried over magnesium sulfate, and the solvent was removed *in vacuo*. The residue was chromatographed (EtOAc/hexane, 1/125), giving the title aldehyde (48.2 g, 78%) as a white solid: ^1H NMR δ 7.92 (m, 2H), 8.39 (s, 1 H), 10.10 (s, 1 H).

METHYL 2-{(BENZOFURAN-5-YL)METHYLENE}-3-OXOBUTYRATE: A mixture of 5-formylbenzofurazan (0.60 g, 4.1 mmol), methyl acetoacetate (0.52 g, 4.5 mmol), piperidine (0.019 g, 0.23 mmol), and acetic acid (0.014 g, 0.23 mmol) in benzene (30 mL) was heated at reflux temperature (equipped with a Dean-Stark trap) for 8 h. Benzene was evaporated *in vacuo*, the residue was dissolved in ethyl acetate (80 mL) and washed with brine (50 mL), saturated potassium bisulfate solution (50 mL), and saturated sodium bicarbonate solution. The ethyl acetate solution was dried over magnesium sulfate, the solvent removed under

reduced pressure and the residue was purified by column chromatography (EtOAc/hexane, 1/9 to 3/20). The desired product was obtained as oil (0.98 g, 98%) and was used in the next step without any further characterization.

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6 - (BENZOFURAZAN-5-YL) -1,6-DIHYDRO-2-METHOXY-5-METHOXCAR
BONYL-4- METHYL PYRIMIDINE: A mixture of methyl
2-{(benzofuran-5-yl)-methylene}-3-oxobutyrate (1.02 g,
4.10 mmol), O-methylisourea hydrogen sulfate (1.06 g, 6.20
mmol), and NaHCO₃ (1.30 g, 16.4 mmol) in DMF (15 mL) was
stirred and heated at 70 °C for 16 h. The mixture was
cooled, diluted with EtOAc (50 mL) and washed with water
(5X 50 mL), brine (50 mL) and dried over magnesium
sulfate. The solvent was evaporated and the crude product
was purified by flash chromatography (EtOAc/hexane, 1/9 to
1/5), giving the desired product as an oil (0.520 g, 43%):
¹H NMR δ 2.38 and 2.42 (2 s, 3 H), 3.60 and 3.66 (2 s, 3 H),
3.74 and 3.82 (2 s, 3 H), 5.53 and 5.68 (2 s, 1 H), 6.31
and 6.32 (br s, 1 H), 7.0-7.8 (m, 3 H).

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6 - (BENZOFURAZAN-5-YL) -1,6-DIHYDRO-2-METHOXY-5-METHOXCAR
BONYL-4- METHYL-1-[(4-NITROPHENYLOXY) CARBONYL] PYRIMIDINE:
T o a s o l u t i o n o f
6-(benzofuran-5-yl)-1,6-dihydro-2-methoxy
-5-methoxycarbonyl-4- methylpyrimidine (0.485 g, 1.6 mmol)
and 4-dimethylaminopyridine (0.200 g, 1.64 mmol) in CH₂Cl₂
(20 mL) at 0-5 °C was added 4-nitrophenyl chloroformate
(0.307 g, 1.52 mmol). The mixture was then allowed to
warm to room temperature. After 12 h, the solvent was
evaporated and the residue was purified by flash
chromatography (EtOAc/hexane, 1/9 to 3/20), giving the
desired product as white crystals (0.665 g, 89%); mp
180-183 °C; ¹H NMR δ 2.54 (s, 3 H), 3.75 (s, 3 H), 3.98 (s,
3 H), 6.37 (s, 1 H), 7.40 (d, J=9.3 Hz, 2 H), 7.52 (d,

J=9.0 Hz, 1 H), 7.68 (s, 1 H), 7.84 (d, J=9.0 Hz, 1 H), 8.32 (d, J=9.3 Hz, 2 H).

(+) and (-)-6-(BENZOFURAZAN-5-YL)-1,6-DIHYDRO-2-METHOXY-5-METHOXYCARBONYL-1-[N-(S)-1-(1-PHENYLETHYL)]-4-METHYLPYRIMIDINE: A solution of 6-(benzofurazan-5-yl)-1,6-dihydro-2-methoxy-5-methoxycarbonyl-4-methyl-1-(4-nitrophenoxy)carbonylpyrimidine (800 mg, 1.71 mmol) and (S)-(-)- α -methylbenzylamine (269 mg, 2.22 mmol) in THF (50 mL) was stirred at room temperature for 12 h. The THF was removed *in vacuo* and the residue was dissolved in EtOAc (100 mL), washed by 10% aqueous K₂CO₃ solution (3x50 mL), brine (50 mL) and dried (Na₂SO₄). After removal of the solvent, the residue was purified by chromatography (EtOAc/hexane, 1/20 to 3/20), separating the two diastereomers. The isomers of 6-(benzofurazan-5-yl)-1,6-dihydro-2-methoxy-5-methoxycarbonyl-1-[N-(S)-1-(1-phenylethyl)]-4-methylpyrimidine were obtained as colorless oils. 1st Isomer (367 mg, 47.7%): [α]_D = +278 (c=0.50, CHCl₃); ¹H NMR δ 1.54 (d, J=6.9 Hz, 3H), 2.45 (s, 3H), 3.68 (s, 3H), 3.99 (s, 3H), 5.02 (quintet, J=6.9 Hz, 1H), 6.71 (s, 1H), 6.89 (d, J=6.6 Hz, 1H), 7.2-7.9 (m, 8H). 2nd Isomer (205 mg, 26.6%): [α]_D = -81 (c=0.43, CHCl₃); ¹H NMR δ 1.52 (d, J=6.6 Hz, 3H), 2.48 (s, 3H), 3.71 (s, 3H), 3.96 (s, 3H), 5.00 (quintet, J=6.6 Hz, 1H), 6.74 (s, 1H), 6.90 (d, J=6.5 Hz, 1H), 7.2-7.9 (m, 8H).

6-(BENZOFURAZAN-5-YL)-1,6-DIHYDRO-2-METHOXY-5-METHOXYCARBONYL-4-METHYLPYRIMIDINE: A solution of the 1st isomer of 6-(benzofurazan-5-yl)-1,6-dihydro-2-methoxy-5-methoxycarbonyl-1-[N-(S)-1-(1-phenylethyl)]-4-methylpyrimidine (960 mg, 2.14 mmol) and 1,8-diazabicyclo[5.4.0]undec-7-ene (107 mg, 0.705 mmol) in

toluene (50 mL) was stirred at 100 °C for 5 h. After cooling to room temperature, toluene was removed *in vacuo* and the residue was purified by chromatography (EtOAc/hexane, 1/9 to 3/7). 6-(Benzofurazan-5-

5 yl)-1,6-dihydro-2-methoxy-

5-methoxycarbonyl- 4-methylpyrimidine was obtained as a colorless oil (635 mg, 98.3%). ^1H NMR δ 2.38 (s, 3H), 3.66 (s, 3H), 3.74 (s, 3H), 5.68 (s, 1H), 6.32 (br s, 1H), 7.0-7.8 (m, 3H).

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6-(BENZOFURAZAN-5-YL)-1,6-DIHYDRO-2-METHOXY-5-METHOXCARBONYL-4-METHYL-1-(4-NITROPHENOXY)CARBONYLPYRIMIDINE: To

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a solution of 6-(benzofuran-5-yl)-1,6-dihydro-2-methoxy-5-methoxycarbonyl- 4-methylpyrimidine (0.485 g, 1.60 mmol) and 4-dimethylamino-pyridine (0.200 g, 1.60 mmol) in CH_2Cl_2 (20 mL), at 0-5 °C, was added 4-nitrophenyl chloroformate (0.307 g, 1.52 mmol). After addition, the mixture was allowed to warm to room temperature. After 12 hours, the solvent was evaporated and the residue was purified by

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flash column chromatography (EtOAc/hexane, 1/9 to 3/20), giving the desired product as white crystals (0.665 g, 89%): mp 180-183 °C; ^1H NMR δ 2.54 (s, 3 H), 3.75 (s, 3 H), 3.98 (s, 3 H), 6.37 (s, 1 H), 7.40 (d, J = 9.3 Hz, 2 H), 7.52 (d, J = 9.0 Hz, 1 H), 7.68 (s, 1 H), 7.84 (d, J = 9.0 Hz, 1 H), 8.32 (d, J = 9.3 Hz, 2 H); $[\alpha]_D$ = +266 ($c=2.70$, CH_2Cl_2).

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METHYL 2-{(3,4-DIFLUOROPHENYL)METHYLENE}-3-OXOBUTYRATE: A mixture of 3,4-difluorobenzaldehyde (14.2 g, 0.100 mol), methyl acetoacetate (12.2 g, 0.105 mol), piperidine (0.430 g, 5 mmol), and acetic acid (0.30 g, 5 mmol) in benzene (150 mL) was stirred and heated at reflux temperature (equipped with a Dean-Stark trap) for 8 h. The benzene was evaporated and the residue was dissolved in ethyl

acetate (200 mL). The resulting solution was washed with brine (50 mL), saturated potassium bisulfate solution (50 mL), and saturated sodium bicarbonate solution. The ethyl acetate solution was dried over magnesium sulfate and the solvent was removed under reduced pressure. The residue was purified by column chromatography (EtOAc/hexane, 1/9 to 3/20), giving the desired product as a yellow oil (9.80 g, 41%) which was used in the subsequent step without any further characterization.

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6-(3,4-DIFLUOROPHENYL)-1,6-DIHYDRO-2-METHOXY-5-METHOXYCARBONYL-4-METHYLPYRIMIDINE: A mixture of methyl 2-{(3,4-difluorophenyl)-methylene}-3-oxobutyrate (8.80 g, 36.3 mmol), O-methylisourea hydrogen sulfate (9.40 g, 546 mmol), and NaHCO₃ (12.3 g, 146 mol) in DMF (30 mL) was heated at 70 °C with stirring for 16 h. The mixture was cooled, diluted with EtOAc (300 mL) and washed with water (5 X 300 mL), brine (300 mL), and dried over magnesium sulfate. The solvent was evaporated and the crude product was purified by flash chromatography (EtOAc/hexane, 1/9 to 3/7) as the gradient eluent, giving the desired product as an oil (3.82 g, 35%).

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6-(3,4-DIFLUOROPHENYL)-1,6-DIHYDRO-2-METHOXY-5-METHOXYCARBONYL-4-METHYL-1-[(4-NITROPHENYLOXY) CARBONYL] PYRIMIDINE: 4-Nitrophenyl chloroformate (1.82 g, 9.04 mmol) was added to a solution of 6-(3,4-difluorophenyl)-1,6-dihydro-2-methoxy-5-methoxycarbonyl-4-methylpyrimidine (2.82 g, 9.46 mmol) and 4-dimethylaminopyridine (1.16 g, 9.52 mmol) in CH₂Cl₂ (50 mL), at 0-5 °C and the mixture was then allowed to warm to room temperature. After 12 h, the solvent was evaporated and the residue was purified by flash chromatography (EtOAc/hexane, 1/9 to 3/20), giving the desired product as white crystals (3.72, 85%): mp 172-174

°C.

6-(3,4-DIFLUOROPHENYL)-1,2,3,6-TETRAHYDRO-2-OXO-5-METHOXYCARBON-YL-4-METHYL-1-(4-NITROPHENOXY)CARBONYLPYRIMIDINE:

5 Aqueous 6 N hydrochloric acid (10 mL) was added to a stirring solution of 6-(3,4-difluorophenyl)-1,6-dihydro-2-methoxy-5-methoxycarbonyl-4-methyl-1(4-nitrophenoxy)carbonylpurimidine (10.0 g) in THF (200 mL) at room temperature. The stirring was
10 continued for 3 h. The solvent was evaporated and the residue was dried under vacuum, giving the desired product as a white powder (9.70 g, 100%): mp 185-186 °C.

15 (+)-1-(3-BROMO-PROPYLCARBAMOYL)-6-(3,4-DIFLUOROPHENYL)-4-METHYL-2-OXO-1,6-DIHYDRO-PYRIMIDINE-5-CARBOXYLIC ACID

METHYL ESTER: A solution of 10% aqueous HCl (5 mL) was added to a stirring solution of (+)-6-(3,4-difluorophenyl)-1,6-dihydro-2-methoxy-5-methoxycarbonyl-4-methyl-1-[
20 (4-nitrophenoxy)-carbonyl]pyrimidine (4.10 g, 9.10 mmol) in THF (20 mL) at room temperature and the resulting solution was stirred overnight. The THF was removed *in vacuo* and the resulting residue was extracted with EtOAc (3 X 20 mL), washed with brine (10 mL) and then dried over Na₂SO₄. The solvent was removed *in vacuo*, giving (+)-6-(3,4-di-fluorophenyl)-1,6-dihydro-2-
25 oxo-5-methoxycarbonyl-4-methyl-1-[
[(4-nitrophenoxy)carbonyl]pyrimidine as a viscous oil (3.8 g, 8.5 mmol). The oil was dissolved in THF (20 mL) and 3-bromo-propylamine hydrobromide (2.33 g, 10.8 mmol) and NaHCO₃ (1.81 g, 21.5 mmol) were added. The resulting suspension was stirred at room temperature overnight. The THF was removed *in vacuo* and the resulting residue was dissolved in water (10 mL) and then extracted with EtOAc (3 X 20 mL). The EtOAc extracts were combined, dried over
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Na_2SO_4 , filtered and the solvent was removed , giving (+)-1-(3-bromo-propylcarbamoyl)-6-(3,4-difluorophenyl)-4-methyl-2-oxo-1,6-dihydropyrimidine-5-carboxylic acid methyl ester (3.28 g, 83%): ^1H NMR δ 2.05-2.15 (m, 2 H), 2.43 (s, 3 H), 3.40-3.56 (m, 4 H), 3.72 (s, 3 H), 6.69 (s, 1 H), 7.08-7.27 (m, 3 H), 7.57 (br s, 1 H), 8.84 (br t, 1 H). Anal. Calcd for $\text{C}_{17}\text{H}_{18}\text{N}_3\text{O}_4 \text{ F}_2\text{Br}$: C, 45.76; H, 4.07; N, 9.42. Found: C, 45.70; H, 3.99; N, 9.16.

10 3-{(3,4,5-TRIFLUOROPHENYL)METHYLENE}-2,4-PENTANEDIONE:
A stirring mixture of 3,4,5-trifluorobenzaldehyde (4.20 g, 26.2 mmol), 2,4-pentanedione (2.62 g, 26.2 mmol), piperidine (0.430 g, 5.00 mmol) in benzene (150 mL) was heated at reflux temperature (equipped with a Dean-Stark trap) for 8 h. The benzene was evaporated and the yellow
15 o i l y r e s i d u e , 2-{(3,4,5-trifluorophenyl)methylene}-2,4-pentanedione, was used in the next step without further purification.

20 6-(3,4,5-TRIFLUOROPHENYL)-1,6-DIHYDRO-2-METHOXY-5-ACETYL
-4-METHYL PYRIMIDINE: A mixture of 2-{(3,4,5-trifluorophenyl)-
methylen}-2,4-pentanedione (26.2 mmol), O-methylisourea hydrogen sulfate (3.22 g, 39.3 mmol), and NaHCO_3 (6.6 g, 78.6 mmol) in EtOH (400 mL) was stirred and heated at 95-100 °C for 6 h. The mixture was filtered and the solid residue was washed with ethanol (100 mL). The solvent was evaporated from the combined filtrates and the crude product was purified by flash column chromatography (EtOAc/hexane, 1/9 to 1/4), giving the desired product as an oil (2.80 g, 36%).

35 6-(3,4,5-TRIFLUOROPHENYL)-1,6-DIHYDRO-2-METHOXY-5-ACETYL
-4-METHYL-1-[(4-NITROPHENYLOXY) CARBONYL] PYRIMIDINE:
4-Nitrophenyl chloroformate (1.89 g, 9.38 mmol) was added

to a solution of 6-(3,4,5-trifluorophenyl)-1,6-dihydro-2-methoxy-5-acetyl-4-meth-ylpyrimidine (2.80 g, 9.38 mmol) and pyridine (10 mL) in CH₂Cl₂ (200 mL) at 0-5 °C, and the resulting mixture was allowed to warm to room temperature.

5 After 12 h, the solvent was evaporated and the residue was purified by flash chromatography (dichloro-methane/EtOAc, 1/9 to 3/20), giving the desired product as a white powder (4.00 g, 92%).

10 6-(3,4,5-TRIFLUOROPHENYL)-1,2,3,6-TETRAHYDRO-2-OXO-5-ACETYL-4-METHYL-1-[(4-NITROPHENYLOXY) CARBONYL] PYRIMIDINE: A solution of 6 N aqueous HCl (4 mL) was added to a stirring solution of 6-(3,4,5-trifluorophenyl)-1,6-dihydro-2-methoxy-5-acetyl-4-methyl-1-[(4-nitrophenyloxy) carbonyl] pyrimidine (4.00 g, 8.63 mmol) in THF (100 mL) at 0-5 °C, and the mixture was allowed to warm to room temperature. After 2 h, solvent was evaporated and the product dried under vacuum. The product was obtained as a pure single component and used in the next step without any further purification (3.88 g, 100%).

25 Procedures for the Synthesis of the Piperidine Intermediates
(reference for the general procedure for Pd coupling of vinyl triflate and boronic acids or tributyl tin reagents:
See, Wuston, Wise *Synthesis* (1991), 993)

Piperidine Side Chain Intermediates

TERT-BUTYL 4-{[(TRIFLUOROMETHYL) SULFONYL] OXY}-1,2,3,6-

5 TETRAHYDRO-1-PYRIDINECARBOXYLATE:

n-Butyl lithium (17.6 mL, 44.2 mmol, 2.5 M in hexanes) was added to a solution of diisopropyl amine (96.2 mL, 44.2 mmol) in 40 mL of dry THF at 0 °C and stirred for 20 minutes. The reaction mixture was cooled to -78 °C and 10 tert-butyl 4-oxo-1-piperidinecarboxylate (Aldrich Chemical Company, 40.0 mmol) in THF (40 mL) was added dropwise to the reaction mixture and stirred for 30 minutes. Tf₂NPh (42.0 mmol, 15.0 g) in THF (40 mL) was added dropwise to the reaction mixture and stirred at °C overnight. The reaction mixture was concentrated in vacuo, re-dissolved in hexanes:EtOAc (9:1), passed through a plug of alumina and the alumina plug was washed with hexanes:EtOAc (9:1). The combined extracts were concentrated to yield 16.5 g of the desired product 15 that was contaminated with some starting Tf₂NPh.

20 ¹H NMR (400 MHz, CDCl₃) δ 5.77 (s, 1 H), 4.05 (dm, 2 H, J=3.0 Hz), 3.63 (t, 2 H, J=5.7 Hz), 2.45 (m, 2 H), 1.47 (s, 9 H).

25 TERT-BUTYL 4-[3-(AMINO) PHENYL]-1,2,3,6-TETRAHYDRO-1-PYRIDINECARBOXYLATE:

A mixture of 2 M aqueous Na₂CO₃ solution (4.2 mL), tert-butyl 4-{[(trifluoromethyl)sulfonyl]oxy}-1,2,3,6-tetrahydro-1-pyridine-carboxylate (0.500 g, 1.51 mmol), 30 3-aminophenylboronic acid hemisulfate (0.393 g, 2.11 mmol), lithium chloride (0.191 g, 4.50 mmol) and tetrakis-triphenylphosphine palladium (0) (0.080 g, 0.075 mmol) in dimethoxyethane (5 mL) was heated at

reflux temperature for 3 hours, under an inert atmosphere (an initial degassing of the mixture is recommended to prevent the formation of triphenylphosphine oxide). The organic layer of the cooled reaction mixture was separated and the aqueous layer was washed with ethyl acetate (3X). The combined organic extracts were dried and concentrated *in vacuo*. The crude product was chromatographed (silica, hexanes:EtOAc:dichloromethane (6:1:1) with 1% added isopropylamine to protect the BOC group from hydrolysis) to give 0.330 g of the desired product in 81% yield:

¹H NMR (400 MHz, CDCl₃) δ 7.12 (t, 1H, J= 7.60 Hz), 6.78 (d, 1H, J= 8.4 Hz), 6.69 (t, 1H, J= 2.0 Hz), 6.59 (dd, 1H, J= 2.2, 8.0 Hz), 6.01 (m, 1H), 4.10-4.01 (d, 2H, J= 2.40 Hz), 3.61 (t, 2H, J= 5.6 Hz), 2.52-2.46 (m, 2H), 1.49 (s, 9H); ESMS m/e : 275.2 (M + H)⁺.
Anal. Calc. for C₁₆H₂₄N₂O₂: C, 70.04; H, 8.08; N, 10.21.
Found: C, 69.78; H, 7.80; N, 9.92.

TERT-BUTYL 4-[3-(AMINO) PHENYL]-1-PIPERIDINECARBOXYLATE
A mixture of 3.10 g of tert-butyl 4-(3-aminophenyl)-1,2,3,6-tetrahydropyridine-1-carboxylate (11.3 mmol) and 1.0 g of 10% Pd/C in 200 mL of ethanol was hydrogenated at room temperature using the balloon method for 2 days. The reaction mixture was filtered and washed with ethanol. The combined ethanol extracts were concentrated *in vacuo* and the residue was chromatographed on silica (dichloromethane: methanol 95:5 with 1% isopropylamine added to protect the BOC group from hydrolysis) to give 2.63 g of the desired product (84%).

**TERT-BUTYL 4-(3-NITROPHENYL)-3,6-DIHYDRO-1(2H)-
PYRIDINECARBOXYLATE**

5 ¹H NMR (400 MHz, CHCl₃) δ 8.23 (s, 1H), 8.11 (d, 1H,
J=8.0 Hz), 7.69 (d, 1H, J=8.0 Hz), 7.51 (t, 1H, J=8.0
Hz), 6.20 (m, 1H), 4.17-4.08 (m, 2H), 3.67 (t, 2H, J=5.6
Hz), 2.61-2.52 (m, 2H), 1.50 (s, 9H); ESMS m/e : 249.1
(M + H - C₄H₈)⁺.

10 **1,2,3,6-TETRAHYDRO-4-(3-NITROPHENYL) PYRIDINE:** Into a
stirred solution of 5.00 g (16.0 mmol) of *tert*-butyl
1,2,3,6-tetrahydro-4-(3-nitrophenyl)pyridine-1-
carboxylate in 100 ml of 1,4-dioxane at 0°C was bubbled
15 HCl gas for 10 minutes. The reaction mixture was
allowed to warm to room temperature and the bubbling of
the HCl gas was continued for an additional 1 hour. The
solvent was removed *in vacuo*, the residue was dissolved
in 50 mL of water and was neutralized by the addition of
20 KOH pellets. The aqueous solution was extracted with 3
X 80 mL of dichloromethane and the combined organic
extracts were dried (MgSO₄), filtered and concentrated *in*
vacuo. The residue was purified by column
chromatography (silica, 9 : 1 ,dichloromethane :
25 methanol + 1% isopropyl amine) to afford 2.85 g (87.5%
yield) of the desired product: ¹H NMR (400 MHz, CDCl₃) δ
8.24 (s, 1H), 8.09 (d, 1H, J=8.4 Hz), 7.71 (d, 1H, J=8.0
Hz), 7.49 (t, 1H, J=8.0 Hz), 6.35-6.25 (m, 1H), 3.58
(apparent q, 2H, J=3.0 Hz), 3.14 (t, 2H, J=5.6 Hz),
30 2.54-2.46 (m, 2H).

**TERT-BUTYL 3-(4-(3-NITROPHENYL)-3,6-DIHYDRO-1(2H)-
PYRIDINYL) PROPYLCARBAMATE:** A mixture of 2.80 g (14.0

mmol) of 1,2,3,6-tetrahydro-4-(3-nitrophenyl)pyridine, 3.60 g (15.0 mmol) of tert-butyl N-(3-bromopropyl)carbamate, 11.6 g (84.0 mmol) of K₂CO₃, 14.6 mL (84.0 mmol) of diisopropylethylamine and 0.78 g (2.00 mmol) of tetrabutylammonium iodide in 250 mL of 1,4-dioxane was heated at reflux temperature for 14 hours. The reaction mixture was filtered and the filtrate was dried (MgSO₄), concentrated in vacuo and the residue was purified by column chromatography (silica, 9:1, dichloromethane: methanol + 1% isopropyl amine) to afford 4.35 g (85.7% yield) of the desired product: ¹H NMR (400 MHz, CDCl₃) δ 8.24 (t, 1H, J=1.9 Hz), 8.09 (dd, 1H, J=1.9, 8.0 Hz), 7.70 (apparent d, 1H, J=8.0 Hz), 7.49 (t, 1H, J=8.0 Hz), 6.23 (m, 1H), 3.29-3.18 (m, 4H), 2.75 (t, 2H, J=5.6 Hz), 2.64-2.54 (m, 4H), 1.82-1.70 (m, 2H), 1.44 (s, 9H); ESMS m/e : 362.2 (M + H)⁺.

3-(4-(3-NITROPHENYL)-3,6-DIHYDRO-1(2H)-PYRIDINYL)-1-PROPANAMINE: Into a stirred solution of 4.35 (12.0 mmol) of tert-butyl 3-(4-(3-nitrophenyl)-3,6-dihydro-1(2H)-pyridinyl)propylcarbamate in 100 ml of 1,4-dioxane at 0°C was bubbled HCl gas for 10 minutes. The reaction mixture was allowed to warm to room temperature and the bubbling was continued for an additional 1 hour. The solvent was removed in vacuo, the residue was dissolved in 50 mL of water and was neutralized by the addition of KOH pellets. The aqueous solution was extracted with 3 X 80 mL of dichloromethane, the combined organic extracts were dried (MgSO₄), filtered and concentrated in vacuo. The residue was purified by column chromatography (silica, 9 : 1 ,dichloromethane : methanol + 1% isopropyl amine) to afford 3.05 g (97.0% yield) of the desired product: ¹H NMR (400 MHz, CDCl₃) δ

8.24 (t, 1H, J=1.8 Hz), 8.09 (dd, 1H, J=1.8, 8.2 Hz),
7.69 (dd, 1H, J=1.8, 8.2 Hz), 7.48 (t, 1H, J=8.2 Hz),
6.24 (m, 1H), 3.21 (d, 2H, J=3.6 Hz), 2.84 (t, 2H, J=6.6
Hz), 2.75 (t, 2H, J=5.8 Hz), 2.64-2.54 (m, 4H), 1.76 (m,
2H); ESMS m/e : 262.2 (M + H)⁺; Anal. Calc. for
5 C₁₄H₁₉N₃O₂ (0.06 CHCl₃): C, 62.90; H, 7.16; N, 15.65.
Found: C, 63.20; H, 7.16; N, 15.65.

METHYL (4S)-3-[({3-[4-(3-AMINOPHENYL)-1-
PIPERIDINYL]PROPYL}AMINO)CARBONYL]-4-(3,4-
DIFLUOROPHENYL)-6-(METHOXYMETHYL)-2-OXO-1,2,3,4-

TETRAHYDRO-5-PYRIMIDINECARBOXYLATE: A mixture of 3.02 g
5 (6.33 mmol) 5-methyl 1-(4-nitrophenyl) (6S)-6-(3,4-
difluorophenyl)-4-(methoxymethyl)-2-oxo-3,6-dihydro-
1,5(2H)-pyrimidinedicarboxylate, 1.50 g (5.80 mmol) of
10 3-(4-(3-nitrophenyl)-3,6-dihydro-1(2H)-pyridinyl)-1-
propanamine, 7.94 g (75.5 mmol) of K₂CO₃ and 1.00 mL of
methanol in 200 mL dichloromethane (under argon) was
stirred at room temperature for 1 hour. The reaction
mixture was filtered and concentrated *in vacuo*. The
residue was dissolved in 100 mL of ethyl acetate and
washed 3 X 50 mL of 5% aqueous NaOH solution, the
15 organic layer was dried (MgSO₄) and concentrated *in vacuo*. The residue was dissolved in 100 mL of anhydrous
ethanol containing 0.50 g 10% Pd/C and the reaction
mixture was stirred under a hydrogen balloon for 24
hours. The reaction mixture was passed through a column
20 of Celite 545 filtering agent, washed with ethanol, the
filtrate was dried (MgSO₄) and concentrated *in vacuo*.
The residue was purified by column chromatography
(silica, 9.5 : 0.5 , dichloromethane : methanol + 1%
isopropyl amine) to afford 1.65 g (52.0% yield) of the
25 desired product.

TERT-BUTYL 4-[3-(ISOBUTYRYLAMINO)PHENYL]-3,6-DIHYDRO-
1(2H)-PYRIDINECARBOXYLATE: Into a solution of 4.00 g
(16.0 mmol) of tert-butyl 4-(3-aminophenyl)-3,6-dihydro-
30 1(2H)-pyridinecarboxylate and 5.60 mL (32.0 mmol) of
diisopropylethylamine in 100 mL dichloromethane was
slowly added 1.90 mL (19.0 mmol) of isobutyryl chloride.
The reaction mixture was stirred at room temperature for

2 hours, washed with water, dried ($MgSO_4$), and concentrated *in vacuo*. The residue was purified by column chromatography (silica, 50 : 46 : 3 : 1, hexanes : dichloromethane : methanol : isopropyl amine) to afford 2.90 g (52.0% yield) of the desired product: 1H NMR (400 MHz, $CDCl_3$) δ 7.69 (s, 1 H), 7.34 (d, 1 H, $J=7.8$ Hz), 7.27 (t, 1H, $J=7.8$ Hz), 7.11 (d, 1H, $J=7.8$ Hz), 6.04 (s, 1H), 4.05 (s, 2H), 3.62 (apparent t, 2 H, $J=4.9$ Hz), 2.51 (m, 3H), 1.49 (s, 9H), 1.25 (d, 6H, $J=7.4$ Hz); ESMS m/e : 345.5 ($M + H$) $^+$. Anal. Calc. for $C_{20}H_{28}N_2O_3 + 0.175 CHCl_3$: C, 66.33; H, 7.77; N, 7.67. Found: C, 66.20; H, 7.41; N, 7.88

TERT-BUTYL 4-[3-(ISOBUTYRYLAMINO)PHENYL]-1-

15 **PIPERIDINECARBOXYLATE:** A mixture of 2.90 g (8.40 mmol) of tert-butyl 4-[3-(isobutyryl amino)phenyl]-3,6-dihydro-1(2H)-pyridinecarboxylate and 0.80 g of 10% yield Pd/C in 100 mL of ethanol was stirred under a hydrogen balloon for 24 hours. The reaction mixture was passed through a column of Celite 545 filtering agent, the filtrate was dried ($MgSO_4$) and concentrated *in vacuo*. The residue was purified by column chromatography (silica, 9.5 : 0.5 , dichloromethane : methanol + 1% isopropyl amine) to afford 2.40 g (84.0% yield) of the desired product: 1H NMR (400 MHz, $CDCl_3$) δ 7.49-7.44 (m, 2H), 7.24 (t, 1H, $J=7.6$ Hz), 6.93 (d, 1H, $J=7.6$ Hz), 4.20-4.10 (m, 2H), 2.86-2.45 (m, 4H), 1.86-1.75 (m, 4H), 1.48 (s, 9H), 1.24 (d, 6H, $J=6.8$ Hz); ESMS m/e : 345.2 ($M + H$) $^+$; Anal. Calc. for $C_{20}H_{30}N_2O_3 + 0.3H_2O$: C, 68.27; H, 8.77; N, 7.96. Found: C, 68.25; H, 8.54; N, 7.84.

2-METHYL-N-[3-(4-PIPERIDINYL)PHENYL]PROPANAMIDE: Into a stirred solution of 2.20 (6.50 mmol) of *tert*-butyl 4-[3-

(isobutyrylamino)phenyl]-1-piperidinecarboxylate in 100 ml of 1,4-dioxane at 0 °C was bubbled HCl gas for 10 minutes. The reaction mixture was allowed to warm to room temperature and the bubbling of the HCl gas was continued for 1 hour. The solvent was removed *in vacuo*, the residue was dissolved in 50 mL of water and was neutralized by the addition of KOH pellets. The aqueous solution was extracted with 3 X 80 mL of dichloromethane, the combined organic extracts were dried ($MgSO_4$), filtered and concentrated *in vacuo*. The residue was purified by column chromatography (silica, 9 : 1 ,dichloromethane : methanol + 1% isopropyl amine) to afford 0.700 g (46.0% yield) of the desired product: 1H NMR (400 MHz, $CDCl_3$) δ 7.47 (s, 1H), 7.40 (d, 1H, $J=7.8$ Hz), 7.24 (t, 1H, $J=7.8$ Hz), 7.00 (d, 1H, $J=7.8$ Hz), 3.23-3.14 (m, 5H), 2.82-2.57 (m, 4H), 1.20 (d, 6H, $J=6.8$ Hz); ESMS m/e : 247.2 ($M + H$) $^+$; The hydrochloride salt was used for the combustion analysis: Anal. Calc. for $C_{15}H_{22}N_2O+HCl+0.15 CHCl_3$: C, 60.51; H, 7.76; N, 9.32. Found: C, 60.57; H, 7.83; N, 8.88.

3-(4-PIPERIDINYL)ANILINE: 1H NMR (400 MHz, $CDCl_3$) δ 7.01 (t, 1H, $J=7.6$ Hz), 6.62-6.54 (m, 3H), 3.16 (br d, 2H, $J=10.3$ Hz), 2.75 (dt, 2H, $J=2.7, 12.3$ Hz), 2.56 (tt, 1H, $J=3.6, 12.3$ Hz), 1.81 (br d, 2H, $J=12.3$ Hz), 1.65 (dq, 2H, $J=4.0, 12.3$ Hz); ESMS m/e : 177.2 ($M + H$) $^+$.

TERT-BUTYL 4-(4-NITROPHENYL)-3,6-DIHYDRO-1(2H)-PYRIDINECARBOXYLATE: To a 25-mL RB flask, equipped with a condenser, was added *tert*-butyl 4-[(trifluoromethyl)sulfonyl]oxy]-3,6-dihydro-1(2H)-pyridinecarboxylate (1.0 g), 4-nitrophenylboronic acid

(0.71 g), sodium carbonate (0.430 mL of 2M solution), lithium chloride (0.382 g), tetrakis(triphenylphosphine)- palladium (0) (0.173 g) and ethylene glycol dimethyl ether (10 mL). The reaction mixture was flushed with Argon three times, then the reaction mixture was heated to 100 °C for 3 hrs. After cooling to room temperature, the reaction mixture was diluted with methylene chloride (30 mL) and water (30 mL) and the organic layer was separated. The aqueous layer was extracted with methylene chloride (3x20 mL) and the combined organic extracts were washed with sat NH₄Cl (20 mL) and brine (20 mL), dried over MgSO₄ and concentrated under reduced pressure. The residue was purified by chromatography (6:1=hexane:ethyl acetate with 1% NH₃) to afford the product (0.55 g, 59.9%) as a yellow oil. The compound is not stable at room temperature and should be used as prompt as practical: ¹H NMR (400 MHz, CDCl₃) δ 8.20 (d, 2H, J=8.6 Hz), 7.51 (d, 2H, J=8.6 Hz), 6.24 (m, 1H), 4.13 (m, 2H), 3.67 (apparent t, 2H, J=5.5 Hz), 2.55 (m, 2H), 1.49 (s, 9H).

4-(4-NITROPHENYL)-1,2,3,6-TETRAHYDROPYRIDINE:

4-(4-Nitrophenyl)-1,2,3,6-tetrahydropyridine was prepared by a similar procedure to that used for the preparation of 2-methyl-N-[3-(4-piperidinyl)phenyl]propanamide using HCl gas and tert-Butyl 4-(4-Nitrophenyl)-3,6-dihydro-1(2H)-pyridinecarboxylate (130 mg) in dioxane (5.0 mL) at room temperature. The reaction mixture was concentrated *in vacuo* to give the crude product (69.8 mg) that used in the next reaction without further purification.

Dihydropyrimidine Intermediates

3-(3,4,5-TRIFLUOROBENZYLIDENE)-2,4-PENTANEDIONE: A

5 stirring mixture of 3,4,5-trifluorobenzaldehyde (4.20 g, 26.2 mmol), 2,4-pentanedione (2.62 g, 26.2 mmol), piperidine (0.430 g, 5.00 mmol) in benzene (150 mL) was heated at reflux temperature in a Dean-Stark apparatus for 8 h. The benzene was evaporated and the yellow oily
10 residue was used in the next step without further purification.

1-[2-METHOXY-4-METHYL-6-(3,4,5-TRIFLUOROPHENYL)-1,6-

DIHYDRO-5-PYRIMIDINYL]ETHANONE: A mixture 3-(3,4,5-trifluorobenzylidene)-2,4-pentanedione (26.2 mmol), O-methylisourea hydrogen sulfate (3.22 g, 39.3 mmol), and NaHCO₃ (6.6 g, 78.6 mmol) in EtOH (400 mL) was stirred and heated at 95-100 °C for 6 h. The mixture was filtered and the solid filter cake was washed with ethanol (100 mL). The solvent was evaporated from the combined filtrates and the crude product was purified by flash column chromatography (EtOAc/hexane, 1/9 to 1/4), to afford the desired product as an oil (2.80 g, 36%).

25 **4-NITROPHENYL 5-ACETYL-2-METHOXY-4-METHYL-6-(3,4,5-TRIFLUOROPHENYL)-1(6H)-PYRIMIDINECARBOXYLATE:**

4-Nitrophenyl chloroformate (1.89 g, 9.38 mmol) was added to a solution of 1-[2-methoxy-4-methyl-6-(3,4,5-trifluorophenyl)-1,6-dihydro-5-pyrimidinyl]ethanone (2.80 g, 9.38 mmol) and pyridine (10 mL) in CH₂Cl₂ (200 mL) at 0-5 °C, and the resulting mixture was allowed to warm to room temperature. After 12 h, the solvent was evaporated and the residue was purified by flash

chromatography (dichloromethane/EtOAc, 1/9 to 3/20), to give the desired product as a white powder (4.00 g, 92%).

5 4-NITROPHENYL 5-ACETYL-4-METHYL-2-OXO-6-(3,4,5-
TRIFLUOROPHENYL)-3,6-DIHYDRO-1(2H)-
PYRIMIDINECARBOXYLATE:

A solution of 6 N aqueous HCl (4 mL) was added to a well-stirred solution of 4-nitrophenyl 5-acetyl-2-methoxy-4-methyl-6-(3,4,5-trifluorophenyl)-1(6H)-pyrimidinecarboxylate (4.00 g, 8.63 mmol) in THF (100 mL) at 0-5 °C, and the mixture was allowed to warm to room temperature. After 2 h, solvent was evaporated and the product dried under vacuum. The product was obtained as a pure single component and used in the next step without further purification (3.88 g, 100%).

: ^1H NMR (DMSO) δ 10.29 (s, 1H), 8.23 (d, 2H, $J=9.1$ Hz), 7.51 (d, 2H, $J=9.1$ Hz), 7.15-7.07 (m, 2H), 6.18 (s, 1H), 2.30 (s, 3H), 2.28 (s, 3H); ESMS m/e: 450.2 ($M + H$) $^+$; Anal. Calc. for $C_{20}H_{14}F_3N_3O_6$: C, 53.46; H, 3.14; N, 9.35. Found: C, 53.26; H, 3.21; N, 9.35.

BENZYL 2-PROPYONYL-3-(3,4,5-TRIFLUOROPHENYL)-2-PROPENOATE. A solution of benzyl propionylacetate (36.3 g, 176 mmol), 3,4-difluorobenzaldehyde (25.0 g, 176 mmol), piperidine (0.86 mL, 9.0 mmol) and acetic acid (0.49 mL, 9.0 mmol) were heated at reflux temperature with removal of water using a Dean-Stark apparatus for 5h. The solvent was removed *in vacuo* and the residue was dissolved in EtOAc. The organic layer was washed with water (100 mL) followed by brine (100 mL) and dried over anhydrous Na₂SO₄. The solvent was evaporated to afford a

pale yellow syrup (60.2 g), which was used in the next step without further purification.

BENZYL 6-(3,4-DIFLUOROPHENYL)-4-ETHYL-2-METHOXY-1,6-

5 **DIHYDRO-5-PYRIMIDINECARBOXYLATE.** A suspension of benzyl 2-propionyl-3-(3,4,5-trifluorophenyl)-2-propenoate (16.0 g, 48.0 mmol), O-methylisourea hydrogen sulfate (16.65 g, 97.02 mmol), NaHCO₃ (16.3 g, 130.2 mmol) in DMF (190 mL) was stirred at 70 °C for 20h. After cooling to room 10 temperature, the reaction mixture was filtered and the filtrate was diluted with EtOAc (300 mL) and then washed with water (4X100 mL), brine (200 mL) and dried over Na₂SO₄. After removal of solvent, the residue was purified by column chromatography (SiO₂, EtOAc/Hexane, 15 10%-30%) to afford benzyl 6-(3,4-difluorophenyl)-4-ethyl-2-methoxy-1,6-dihydro-5-pyrimidinecarboxylate as a colorless oil (10.6 g, 58% yield). The product was directly used in the next step after ¹H NMR spectroscopy which showed it to be a mixture of amine/imine 20 tautomers.

5-BENZYL 1-(4-NITROPHENYL) 6-(3,4-DIFLUOROPHENYL)-4-ETHYL-2-METHOXY-1,5(6H)-PYRIMIDINEDICARBOXYLATE.

25 Into a well-stirred solution of benzyl 6-(3,4-difluorophenyl)-4-ethyl-2-methoxy-1,6-dihydro-5-pyrimidinecarboxylate (27.5 g, 68.75 mmol) and pyridine (9.2 mL) in CH₂Cl₂ (300 mL) was added 4-nitrophenyl chloroformate (14.49 g, 82.5 mmol) at room temperature. The reaction mixture was stirred for 4 h and then washed 30 with 10% aqueous KOH solution (2 X 150 mL). The organic layer was separated and dried over Na₂SO₄. The solvent was removed *in vacuo* and the residue was used in the next step without further purification: ¹H NMR (CDCl₃) δ

1.24 (t, $J=7.2$ Hz, 3H), 2.81-2.98 (m, 3H), 3.97 (s, 3H),
5.14 (AB_q, 2H), 6.28 (s, 3H), 7.03-7.29 (m, 8H), 7.35 (d,
 $J=9.2$ Hz, 2H), 8.26 (d, $J=9.2$ Hz, 2H).

5 **BENZYL 6-(3,4-DIFLUOROPHENYL)-4-ETHYL-2-METHOXY-1-**
 ($\{[(1R)-1\text{-PHENYLETHYL}]AMINO\}CARBONYL$)**-1,6-DIHYDRO-5-**
PYRIMIDINECARBOXYLATE.

10 Into a stirred mixture of 5-benzyl 1-(4-nitrophenyl) 6-(3,4-difluorophenyl)-4-ethyl-2-methoxy-1,5(6H)-pyrimidinedicarboxylate (12.6 g, 22.86 mmol) in THF (150 mL) was added a solution of R-(+)- α -methyl benzylamine (3.53 mL, 27.44 mmol) at room temperature. The stirring was continued for 12 h and the solvent was removed *in vacuo*. The yellow residue was dissolved in chloroform (200 mL) and was washed with 10% K₂CO₃ solution (2 x 30 mL). The organic layer was dried over Na₂SO₄, filtered and the solvent was removed *in vacuo*. The resulting mixture of diastereomers was separated by column chromatography over silica gel with 9:1 pet. ether:ether to 4:1 pet. ether:ether. First major product to elute was (+)-benzyl 6-(3,4-difluorophenyl)-4-ethyl-2-methoxy-1-($\{[(1R)-1\text{-phenylethyl}]amino\}carbonyl$)-1,6-dihydro-5-pyrimidinedicarboxylate: Colorless oil, R_f= 0.31 (4:1 pet ether:ether); wt.= 3.8 g (60% yield); [α]_D = +267.05 (c = 0.76, CHCl₃); ¹H NMR (CDCl₃) δ 1.22 (t, $J=7.5$ Hz, 3H), 1.52 (d, $J=6.9$ Hz, 3H), 2.88 (q, $J=6.0$ Hz, 2H), 3.99 (s, 3H), 4.99 (m, 1H), 5.09 (AB_q, 2H), 6.66 (s, 1H), 6.99-7.36 (m, 13H); The second major product to elute was (-)-benzyl 6-(3,4-difluorophenyl)-4-ethyl-2-methoxy-1-($\{[(1R)-1\text{-phenylethyl}]amino\}carbonyl$)-1,6-dihydro-5-pyrimidinedicarboxylate: Colorless oil; R_f= 0.22 (4:1 pet ether:ether); wt.= 3.2 g (51.2% yield); [α]_D = -146.89 (c = 0.38, CHCl₃); ¹H NMR (CDCl₃) δ 1.22 (t, $J=7.2$ Hz,

3H), 1.49 (d, $J=6.6$ Hz, 3H), 2.88 (q, $J=6.0$ Hz, 2H), 3.94 (s, 3H), 5.03 (m, 1H), 5.11 (AB_q, 2H), 6.68 (s, 1H), 6.91-7.34 (m, 13H).

5 **(+)-BENZYL 6-(3,4-DIFLUOROPHENYL)-4-ETHYL-2-METHOXY-1,6-DIHYDRO-5-PYRIMIDINECARBOXYLATE.** Into a stirred solution of (+)-benzyl 6-(3,4-difluorophenyl)-4-ethyl-2-methoxy-1-({[(1*R*)-1-phenylethyl]amino}carbonyl)-1,6-dihydro-5-pyrimidinecarboxylate (17.1 mmol, 9.35 g) in CH₂Cl₂ was added 1,8-diazabicyclo[5,4,0]-undec-7-ene (17.1 mmol, 2.56 mL) and stirring was continued for 16 h at room temperature. The solvent was evaporated and the residue was purified by flash column chromatography on silica gel with 3:1 EtOAc/Hexanes as the eluting system. 5.27 g of the (+)-benzyl 6-(3,4-difluorophenyl)-4-ethyl-2-methoxy-1,6-dihydro-5-pyrimidinecarboxylate was obtained (77% yield).

20 **(+)-5-BENZYL 1-(4-NITROPHENYL) 6-(3,4-DIFLUOROPHENYL)-4-ETHYL-2-METHOXY-1,5(6*H*)-PYRIMIDINEDICARBOXYLATE.** Into a well-stirred solution of (+)-benzyl 6-(3,4-difluorophenyl)-4-ethyl-2-methoxy-1,6-dihydro-5-pyrimidinecarboxylate (6.4 g, 16.0 mmol) and pyridine (1.5 mL) in CH₂Cl₂ (150 mL) was added 4-nitrophenyl chloroformate (3.41 g, 19.2 mmol) at room temperature. The reaction mixture was stirred for 4 h and then it was washed with 10% aqueous KOH solution (2 X 100 mL). The organic layer was separated and dried over Na₂SO₄. The solvent was removed *in vacuo*. The residue of (+)-5-benzyl 1-(4-nitrophenyl) 6-(3,4-difluorophenyl)-4-ethyl-2-methoxy-1,5(6*H*)-pyrimidinedicarboxylate was used in the next step without further purification.

a. 2-(4-METHOXYBENZYL)-2-THIOPSEUDOUREA HYDROCHLORIDE.

Into a well-stirred suspension of thiourea (7.6 g, 0.1 mol) in THF (50 mL) at 0 °C, 4-methoxybenzyl chloride (16 g, 0.1 mol) was added in 10 min and the reaction mixture was allowed to warm to room temperature. After 2 hours the reaction mixture was heated to 65 °C and kept at that temperature for 5 hours. The reaction mixture was cooled to room temperature and diluted with diethyl ether (200 mL). The white precipitate that formed was filtered and dried (22.5 g, 96% yield); m. p. 161-163 °C.

b. METHYL 2-{(4-NITROPHENYL)METHYLENE}-3-OXOBUTYRATE.

A mixture of 4-nitrobenzaldehyde (15.1 g, 0.1 mol), methyl acetoacetate (12.773 g, 0.11 mol), piperidine (0.41 g, 4.80 mmol), and acetic acid (0.288 g, 4.8 mmol) in 2-propanol (400 mL) was stirred at room temperature for 48 hours. The resulting white solid, methyl 2-{(4-nitrophenyl)methylene}-3-oxobutyrate was filtered, washed with 2-propanol (2 X 50 mL) and dried (21.8 g, 93% yield).

c.

1,6-DIHYDRO-5-METHOXCARBONYL-2-[{(4-METHOXYPHENYL)METHYL}THIO]-4-METHYL-6-(4-NITROPHENYL)PYRIMIDINE.

A mixture of methyl 2-{(4-nitrophenyl)methylene}-3-oxobutyrate (8.96 g, 0.04 mol), 2-(4-methoxybenzyl)-2-thiopseudourea hydrochloride (9.28 g, 0.04 mol), and NaOAc (3.28 g, 0.04 mol) in DMF (100 mL) was stirred and heated at 70-75 °C for 4.5 hours. The reaction mixture was cooled to room temperature, poured into ice-water (300 mL) and extracted with EtOAc (2 X 400 mL). The combined EtOAc extracts were washed with 10% NaHCO₃ solution (2 X 60 mL), brine (100 mL), and then dried

(MgSO₄). The solvent was evaporated and the crude product was purified by flash column chromatography on silica gel using 10% through 30% EtOAc in hexane as the gradient eluent. The desired product was obtained as an oil, which on trituration with EtOAc/hexane became a yellow solid (11.4 g, 66.7% yield) which was shown by ¹H NMR to be a mixture of tautomers: m.p. 138-139 °C; ¹H NMR (CDCl₃) δ 2.15 (s, 3 H), 3.62 (s, 3 H), 3.72 (s, 3 H), 4.05 and 5.78 (s and d, J=3 Hz, 1 H), 4.08, 4.20 (AB q, J=12.5 Hz, 2 H), 4.21 and 6.40 (s and d, J=3 Hz, 1 H), 6.66 (2 d, J=8.5 Hz, 2 H), 7.08 (2 d, J=8.5 Hz, 2 H), 7.37 (2 d, J=8.8 Hz, 2 H), 8.7 (2 d, J=8.8 Hz, 2 H); Anal. Calcd. for C₂₁H₂₁N₃O₅S: C, 59.00; H, 4.95; N, 9.83. Found: C, 59.02; H, 4.93; N, 9.77.

15

d. 1,6-DIHYDRO-5-METHOXCARBONYL-2-[{(4-METHOXYPHENYL)METHYL}THIO]-4-METHYL-6-(4-NITROPHENYL)-1-[(4-NITROPHENYLOXY)CARBONYL]PYRIMIDINE.

20 Into a well-stirred mixture of 1,6-dihydro-5-methoxy carbonyl-2-[{(4-methoxyphenyl)methyl}thio]-4-methyl-6-(4-nitrophenyl)pyrimidine (4.50 g, 10.5 mmol), NaHCO₃ (3.69 g, 0.044 mol), CH₂Cl₂ (200 mL), and water (50 mL) at 0-5 °C, 4-nitrophenyl chloroformate (2.40 g, 12.0 mmol) was added over a 5 min period and the reaction mixture was allowed to warm to room temperature. After 10 hours, the TLC analysis of the reaction mixture showed the presence of a small amount of starting pyrimidine, therefore, more 4-nitrophenyl chloroformate (0.65 g, 0.0032 mol) was added and the stirring was continued for an additional 4 hours. The two layers were separated, the CH₂Cl₂ layer was washed with saturated aqueous NaHCO₃ solution (3 X 50 mL), dried (MgSO₄), and the solvent evaporated. The residue was recrystallized from CH₂Cl₂

and hexane to give the product as white crystals (5.50 g, 88.4% yield): m.p. 156-157 °C; $^1\text{H-NMR}$ (CDCl_3) δ 2.53 (s, 3 H), 3.70 (s, 3 H), 3.81 (s, 3 H), 4.06, 4.36 (ABq, $J=13.5$ Hz, 2 H), 6.30 (s, 1 H), 6.78 (d, $J=8.6$ Hz, 2 H), 7.17 (d, $J=8.6$ Hz, 2 H), 7.20 (d, $J=8.8$ Hz, 2 H), 7.32 (d, $J=8.8$ Hz, 2 H), 7.97 (d, $J=8.8$ Hz, 2 H), 8.25 (d, $J=8.8$ Hz, 2 H); Anal. Calcd. for $\text{C}_{28}\text{H}_{24}\text{N}_4\text{O}_9\text{S}$: C, 56.75; H, 4.08; N, 9.45. Found: C, 56.49; H, 4.28; N, 9.25.

10 a. 6-(BENZOFURAZAN-5-YL)-1,6-DIHYDRO-2-OXO-5-METHOXCARBONYL-4-BROMOMETHYL-1-[(4-NITROPHENYLOXY)CARBONYL] PYRIMIDINE.

15 Into a well-stirred solution of 6-(benzofurazan-5-yl)-1,6-dihydro-2-methoxy-5-methoxycarbonyl-4-methyl-1-[(4-nitrophenyl-oxy)carbonyl]pyrimidine (0.310 mmol, 0.140 g) in 1.5 mL of chloroform was added a solution of bromine (0.310 mmol, 0.020 mL) in 1.5 mL of chloroform at 0 °C and the solution was allowed to attain room temperature over 1.5 h. The solvent was removed *in vacuo* and the residue was again dissolved in CHCl_3 (10 mL) and washed with brine. The organic layer was separated, dried over Na_2SO_4 , filtered and the solvent was removed *in vacuo* to obtain 0.15 g (88% yield) of 6-(benzofurazan-5-yl)-1,6-dihydro-2-oxo-5-methoxycarbonyl-4-bromomethyl-1-[(4-nitrophenyl-oxy)carbonyl]pyrimidine as a yellow foam. The crude product was used in the next step without purification. $^1\text{H NMR}$ (CDCl_3) δ 3.79 (s, 3 H), 4.72 (ABq, 2 H), 6.47 (s, 1 H), 7.37 (d, $J=9.1$ Hz, 2 H), 7.51 (d, $J=7.8$ Hz, 1 H), 7.80 (s, 1 H), 7.92 (d, $J=9.1$ Hz, 1 H), 8.30 (d, $J=9.1$ Hz, 2 H).

c. 4-NITROPHENYL 4-(2,1,3-BENZOXADIAZOL-5-YL)-2,5-DIOXO-
1,2,5,7-TETRAHYDROFURO[3,4-d]PYRIMIDINE-3(4H)-
CARBOXYLATE.

6-(3,4-Benzofurazan-5-yl)-1,6-dihydro-2-oxo-5-methoxy-
5 carbonyl-4-bromomethyl-1-[(4-
nitrophenyloxy)carbonyl]pyrimidine (0.27 mmol, 0.15 g)
was heated in oil bath for 3 h (bath temperature 130 °C).
The brownish-yellow residue thus obtained was washed
with CHCl₃ and 4-nitrophenyl 4-(2,1,3-benzoxadiazol-5-
10 yl)-2,5-dioxo-1,2,5,7-tetrahydrofuro[3,4-d]pyrimidine-
3(4H)-carboxylate was obtained as an off-white solid
which was used in the next step without further
purification (crude wt. 0.11 g, 93% yield): ¹H NMR (DMSO-
d₆) δ 8.38-7.56 (m, 7H), 6.33 (s, 1H), 5.02 (s, 2H);
15 Anal. Calc. for C₁₉H₁₁N₅O₈+2.3H₂O: C, 47.85; H, 3.28; N,
14.63. Found: C, 47.73; H, 2.51; N, 14.77.

20 5-METHYL 1-(4-NITROPHENYL) 4-(BROMOMETHYL)-6-(3,4-
DIFLUOROPHENYL)-2-OXO-3,6-DIHYDRO-1,5(2H)-
PYRIMIDINEDICARBOXYLATE: Into a well-stirred solution of
25 6-(3,4-Difluorophenyl)-1,6-dihydro-2-methoxy-5-
methoxycarbonyl-4-methyl-1-[(4-
nitrophenyloxy)carbonyl]pyrimidine (1.5 mmol, 0.66 g) in
5 mL of chloroform was added a solution of bromine (1.5
mmol, 0.09 mL) in 3 mL of chloroform at 0 °C and the
solution was allowed to attain room temperature over 1.5
h. The solvent was removed *in vacuo* and the residue was
again dissolved in CHCl₃ (20 mL) and washed with brine.
30 The organic layer was separated, dried over Na₂SO₄,
filtered and the solvent was removed *in vacuo* to afford
the desired product as a yellow foam, which was used in
the next step without purification. ¹H NMR δ 3.75 (s, 3

H), 4.67 (ABq, 2 H), 6.35 (s, 1 H), 7.09-7.19 (m, 4 H),
7.37 (d, J=9.0 Hz, 2 H), 8.27 (d, J=9.0 Hz, 2 H).

4-NITROPHENYL 4-(3,4-DIFLUOROPHENYL)-2,5-DIOXO-1,2,5,7-TETRAHYDROFURO[3,4-D]PYRIMIDINE-3(4H)-CARBOXYLATE.

5 5-methyl 1-(4-nitrophenyl) 4-(bromomethyl)-6-(3,4-difluorophenyl)-2-oxo-3,6-dihydro-1,5(2H)-pyrimidinedicarboxylate (1.5 mmol, 0.81 g) was heated in an oil bath for 3 h (bath temperature 130 °C). The brown residue thus obtained was washed with CHCl₃, and the desired product was obtained as a pale brown solid which was used in the next step without further purification (crude wt. 0.51 g): ¹H NMR (DMSO-d₆) δ 4.94 (br s, 2 H), 6.08 (s, 1 H), 7.20-7.43 (m, 4 H), 8.35 (d, J=10.2 Hz, 2 H).

4-NITROPHENYL 4-(1,3-BENZODIOXOL-5-YL)-2,5-DIOXOHEXAHYDROFURO[3,4-D]PYRIMIDINE-3(4H)-CARBOXYLATE: ¹H NMR (DMSO) δ 11.35 (s, 1H), 8.16 (d, 2H, J=9.5 Hz), 7.32 (d, 2H, J=8.9 Hz), 6.81-6.65 (m, 3H), 5.88 (s, 1H), 4.85 (ABq, 2H); ESMS m/e : 440.1 (M + H)⁺; Anal. Calc. for C₂₀H₁₅N₃O₉+1.5H₂O: C, 51.29; H, 3.87; N, 8.97. Found: C, 51.38; H, 2.85; N, 8.73.

25 **5-METHYL 1-(4-NITROPHENYL) (6S)-6-(3,4-DIFLUOROPHENYL)-4-METHYL-2-OXO-3,6-DIHYDRO-1,5(2H)-PYRIMIDINEDICARBOXYLATE:** ¹H NMR (400 MHz, CDCl₃) δ 8.29 (d, 2H, J=9.1 Hz), 7.36 (d, 2H, J=8.9 Hz), 7.25-7.11 (m, 3H), 6.37 (s, 1H), 3.75 (s, 3H), 2.46 (s, 3H); ESMS m/e: 448.1 (M + H)⁺; Anal. Calc. for C₂₀H₁₅F₂N₃O₇: C, 53.70; H, 3.38; N, 9.39. Found: C, 53.35; H, 3.36; N, 9.27.

General Procedure for the reaction of pyrimidine-3-carboxylic acid-4-nitrophenyl esters with amines:

A solution of substituted pyrimidine-3-carboxylic acid-4-nitrophenyl ester ((0.29 mmol) and a substituted 4-phenyl-1-(3-propylaminopiperidine (0.30 mmol) in 10 mL of anhydrous THF was stirred overnight at room temperature. The solvent was removed *in vacuo* and the residue was purified by column chromatography.

T E R T - B U T Y L 4 - { [(T R I F L U O R O M E T H Y L) S U L F O N Y L] O X Y } - 1 , 2 , 3 , 6 - T E T R A - H Y D R O - 1 -
5 P Y R I D I N E C A R B O X Y L A T E : *n* - B u t y l l i t h i u m (1 7 . 6 m L , 4 4 . 2 m m o l ,
2 . 5 M i n h e x a n e s) w a s a d d e d t o a s o l u t i o n o f d i i s o p r o p y l
1 0 a m i n e (9 6 . 2 m L , 4 4 . 2 m m o l) i n 4 0 m L o f d r y T H F a t 0 ° C a n d
s t i r r e d f o r 2 0 m i n u t e s . T h e r e a c t i o n m i x t u r e w a s c o o l e d
1 5 t o - 7 8 ° C a n d t e r t - b u t y l 4 - o x o - 1 - p i p e r i d i n e c a r b o x y l a t e
v a c u o , r e - d i s s o l v e d i n h e x a n e s / E t O A c (9 / 1) , p a s s e d t h r o u g h
2 0 a p l u g o f a l u m i n a a n d w a s h e d w i t h h e x a n e s / E t O A c (9 / 1) .
T h e c o m b i n e d e x t r a c t s w e r e c o n c e n t r a t e d t o y i e l d 1 6 . 5 g o f
2 5 t h e d e s i r e d p r o d u c t t h a t w a s c o n t a m i n a t e d w i t h a s m a l l
a m o u n t o f T f 2 N p h . ¹H N M R δ 5 . 7 7 (s , 1 H) , 4 . 0 5 (d m , 2 H ,
J = 3 . 0 H z) , 3 . 6 3 (t , 2 H , J = 5 . 7 H z) , 2 . 4 5 (m , 2 H) , 1 . 4 7
2 0 (s , 9 H) .

T E R T - B U T Y L 4 - [3 - (A C E T Y L A M I N O) P H E N Y L] - 1 , 2 , 3 , 6 - T E T R A H Y D R O - 1 -
2 5 P Y R I D I N E C A R B O X Y L A T E : A m i x t u r e o f s a t u r a t e d o f a q u e o u s
N a 2 C O 3 s o l u t i o n (2 5 m L) , t e r t - b u t y l
4 - { [(t r i f l u o r o m e t h y l) s u l f o n y l] o x y } - 1 , 2 , 3 , 6 - t e t r a h y d r o - 1 -
3 0 p y r i d i n e - c a r b o x y l a t e (2 0 m m o l) , 3 - a c e t - a m i d o p h e n y l b o r o n i c
a c i d (3 0 m m o l) a n d t e t r a k i s - t r i p h e n y l p h o s p h i n e p a l l a d i u m
(0) (1 . 1 5 g) a n d d i m e t h o x y e t h a n e (4 0 m L) w a s h e a t e d a t
r e f l u x t e m p e r a t u r e o v e r n i g h t . T h e o r g a n i c l a y e r o f t h e
c o o l e d r e a c t i o n m i x t u r e w a s s e p a r a t e d a n d t h e a q u e o u s
l a y e r w a s w a s h e d w i t h e t h y l a c e t a t e (3 X) . T h e c o m b i n e d
o r g a n i c e x t r a c t s w e r e d r i e d a n d c o n c e n t r a t e d i n v a c u o .
T h e c r u d e p r o d u c t w a s c h r o m a t o g r a p h e d , g i v i n g t h e d e s i r e d

product ¹H NMR δ 8.11 (br s, 1 H), 7.57 (br s, 1 H), 7.41
5 (br δ, 1 H, J=7.8 Hz), 7.25 (apparent t, 1 H, J=7.8 Hz),
7.08 (br d, 1 H, J=7.8 Hz), 5.99 (b s, 1 H), 4.03 (br m,
2 H, J=2.7 Hz), 3.59 (t, 2 H, J=5.7 Hz), 2.46 (m, 2 H,),
2.16 (s, 3 H), 1.49 (s, 9 H).

N1-[3-(1,2,3,6-TETRAHYDRO-4-PYRIDINYL)PHENYL]ACETAMIDE:
A solution of 4 M HCl in dioxane (10 mL) was added to
10 tert-butyl 4-[3-(acetylamino)phenyl]-1,2,3,6-tetrahydro-
1-pyridinecarboxyl-ate (8.25 mmol) in dichloromethane (30
mL). The reaction mixture was stirred at room temperature
overnight, concentrated *in vacuo*, giving the desired
product as the hydrochloride salt (2.1 g). ¹H NMR δ
15 7.41-7.00 (m, 4 H), 6.10 (br, 1 H), 3.55 (m, 2 H), 3.16
(t, 2 H, J = 5.7 Hz), 2.44 (m, 2 H), 2.19 (s, 3 H).

TERT-BUTYL N-(3-BROMOPROPYL)CARBAMATE: Prepared from
3-bromopropylamine hydrobromide and BOC₂O in the presence
of base in dichloromethane: ¹H NMR δ 5.07 (br, 1 H), 3.31
20 (t, 2 H, J=6.6 Hz), 3.12 (apparent br q, 2 H, J=6.0 Hz),
1.92 (p, 2 H, J=6.6 Hz), 1.30 (s, 9H).

REACTION OF N1-[3-(1,2,3,6-TETRAHYDRO-4-PYRIDINYL)PHENYL]
ACETAMIDE WITH TERT-BUTYL N-(3-BROMOPROPYL)CARBAMATE
25
TERT-BUTYL N-(3-{4-[3-(ACETYLAMINO)PHENYL]
-1,2,3,6-TETRAHYDRO-1-PYRIDINYL}PROPYL)CARBAMATE: A
solution of N1-[3-(1,2,3,6-tetrahydro-
4-pyridinyl)phenyl]acetamide hydrochloride (8.24 mmol),
30 tert-butyl N-(3-bromopropyl)carbamate and potassium
carbonate (33 mmol) in dry dioxane (30 mL) was heated at
reflux temperature overnight. The solids were removed by
filtration, the solution was concentrated *in vacuo* and the
product was chromatographed, giving the desired product

(110 mg). ^1H NMR δ 7.65 (s, 1 H), 6.98 (s, 1 H), 7.45 (d, 1 H, $J=7.8$ Hz), 7.16 (apparent t, 1 H, $J=7.8$ Hz), 7.10 (d, 1 H, $J=7.8$ Hz), 6.02 (s, 1 H), 5.23 (b, 1 H), 3.40 (b, 2 H), 3.30-1.80 (m, 10 H), 2.18 (s, 3 H), 1.45 (s, 9 H).

5

Deprotection of BOC:

N1-{3-[1-(3-AMINOPROPYL)-1,2,3,6-TETRAHYDRO-4-PYRIDINYL]PHENYL}ACETAMIDE: A 1:1 solution of TFA:CH₂Cl₂ (5 mL) was added to tert-butyl N-(3-{4-[3-(acetylamino)

10 phenyl]-1,2,3,6-tetrahydro-1-pyridinyl}propyl)carbamate in dichloromethane (5 mL). The resulting solution was stirred at room temperature for 1-3 days, saturated NaHCO₃ was added until pH > 6, the organic layer was separated, and dried *in vacuo*, giving the desired product (45 mg):

15 ^1H NMR δ 7.68 (br, 1 H), 7.35 (dm, 1 H, $J=7.8$ Hz), 7.25 (apparent t, 1 H, $J=7.8$ Hz), 7.15 (dm, 1 H, $J=7.8$ Hz), 6.12 (m, 1 H), 3.22 (m, 2 H), 3.03 (t, 2 H, $J=7.3$ Hz), 2.78 (t, 2 H, $J=5.5$ Hz), 2.70-2.50 (m, 4 H), 2.10 (s, 3 H), 1.87 (p, 2 H, $J=7.3$ Hz).

20

T	E	R	T	-	B	U	T	Y	L
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4-[3-(ACETYLAMINO)PHENYL]-1-PIPERIDINECARBOXYLATE: A

mixture tert-butyl 4-[3-(acetylamino)phenyl]-1,2,3,6-tetrahydro-1-pyridinecarboxylate (710 mg) and 5%

25 Pd/C (100 mg) in EtOH (10 mL) was hydrogenated (balloon technique) at room temperature overnight. The reaction mixture was passed through a pad of Celite 545 and the pad of Celite was washed with ethanol. The combined ethanol extracts were concentrated and chromatographed, giving the

30 desired product (660 mg). ^1H NMR δ 7.80 (s, 1 H), 7.41-7.20 (m, 3 H), 6.94 (d, 1 H, $J=7.5$ Hz), 4.21 (m, 2 H), 2.75 (m, 2 H), 2.62 (m, 1 H), 2.16 (s, 3 H), 1.78 (m, 2 H), 1.56 (m, 2 H), 1.48 (s, 9 H).

N1-[3-(4-PIPERIDYL)PHENYL]ACETAMIDE: A solution of HCl in dioxane (4N, 5 mL) was added to tert-butyl 4-[3-(acetylamino)-phenyl]-1-piperidinecarboxylate (660 mg) in dry dichloromethane (15 mL). The reaction mixture was stirred at room temperature overnight and concentrated *in vacuo*, giving the desired product (550 mg): mp 102-104 °C; ¹H NMR δ 2.02 (d, J=13.2 Hz, 2H), 2.11-2.45 (m, 5H), 2.67-2.77 (m, 1H), 3.00-3.10 (m, 2H), 3.51 (d, J=10.5 Hz, 2H), 6.94 (d, J=7.5 Hz, 1H), 7.20-7.46 (m, 3H), 7.60 (s, 1H).

TERT-BUTYL N-(3-{4-[3-(ACETYLAMINO)PHENYL]PIPERIDINO}PROPYL)-CARBAMATE: A solution of N1-[3-(4-piperidyl)phenyl]acetamide (550 mg, 0.210 mmol), tert-butyl N-(3-bromopropyl)-carbamate (550 mg, 0.230 mmol), K₂CO₃ (1.10 g, 0.890 mmol), diisopropylethyl amine (1.50 mL) and a few crystals of KI in dioxane (20 mL) was heated at reflux temperature for 2 days. The precipitated salts were removed by filtration, concentrated *in vacuo* and the crude product was chromatographed, giving the desired product (340 mg). ¹H NMR δ 8.15 (s, 1 H), 7.47-7.44 (m, 2 H), 7.22 (t, 1 H, J=7.8 Hz), 6.94 (d, 1 H, J=7.8 Hz), 5.53 (b, 1 H), 3.23 (b, 6 H), 2.80-1.60 (m, 9 H), 2.20 (s, 3 H), 1.45 (s, 9 H).

25

N1-{3-[1-(3-AMINOPROPYL)-4-PIPERIDYL]PHENYL}ACETAMIDE: TFA (1.0 mL) was added to a solution of tert-butyl N-(3-{4-[3-(acetylamino)phenyl]piperidino}propyl)carbamate (340 mg) in dry dichloromethane (10 mL) and stirred at room temperature for 5 h. A 10% aqueous solution of KOH was added to the reaction mixture until pH > 6 and then the dichloromethane was removed *in vacuo*. The aqueous layer was frozen and

lyophilized, giving a solid which was then extracted with methanol. Removal of methanol gave the desired product (120 mg) as an oil. ^1H NMR δ 8.56 - 8.46 (s, 1H), 7.43 - 7.30 (m, 2H), 7.23 - 7.16 (apparent t, 1H, $J=7.5$ Hz), 6.95 - 6.92 (m, 1H), 3.03 - 2.99 (m, 2H), 2.77 - 2.73 (t, 2H, $J = 6.6$ Hz), 2.50-1.60 (m, 10 H), 2.13 (s, 3 H).

1-BENZYL-4-HYDROXY-4-(4-FLUORO-2-METHYLPHENYL)PIPERIDINE:
10 ^1H NMR δ 7.40-7.26 (m, 5 H), 6.91-6.76 (m, 3 H), 3.57 (s, 2 H), 2.83-2.72 (m, 2 H), 2.61 (s, 3 H), 2.58-2.43 (m, 2 H), 2.23-2.12 (m, 2 H).

1-BENZYL-4-(4-FLUORO-2-METHYLPHENYL)-1,2,3,6-TETRAHYDRO PYRIDINE: ^1H NMR δ 7.41-7.26 (m, 5 H), 7.05 (dd, 1 H, $J=6.0$, 8.1 Hz), 6.87-6.80 (m, 2 H), 5.52-5.50 (m, 2 H), 3.65 (s, 2 H), 3.13 (q, 2 H, $J=3.3$ Hz), 2.69-2.66 (t, 2 H, $J=5.1$ Hz), 2.35-2.31 (m, 2 H), 2.27 (s, 3 H).

20 4-(4-FLUORO-2-METHYLPHENYL)PIPERIDINE: ^1H NMR δ 7.17 (t, 1 H, $J=7.2$ Hz), 6.83-6.80 (m, 2 H), 3.22 (m, 2 H), 2.81-2.73 (m, 2 H), 2.66 (br s, 1 H), 2.33 (s, 3 H), 1.80-1.60 (m, 4 H).

25 1-BENZYL-4-(3,4,5-TRIFLUOROPHENYL)-1,2,3,6-TETRAHYDRO PYRIDINE: ^1H NMR δ 7.50-7.20 (m, 7 H), 5.67 (m, 1 H), 3.69 (s, 2 H), 3.19 (apparent q, 2 H, $J=2.7$ Hz), 2.75 (t, 2 H, $J=5.7$ Hz), 2.34 (m, 2 H).

30 4-(3,4,5-TRIFLUOROPHENYL)PIPERIDINE: mp 197-199 °C; ^1H NMR δ 2.05 (d, $J=13.2$ Hz, 2H), 2.33 (dd, $J=25.5$ Hz, $J=12.9$ Hz, 2H), 3.06-3.23 (m, 3H), 3.73 (d, $J=12.0$ Hz, 2H), 6.94-7.04 (m, 2H).

4-(3,4,5-TRIFLUOROPHENYL)PIPERIDINE: ^1H NMR δ 7.20-6.80 (m,

2 H), 3.73 (m, 2 H), 3.14 (m, 3 H), 2.33 (m, 2 H), 2.05 (m, 2 H).

T E R T - B U T Y L
5 N-3-[4-(3,4,5-TRIFLUOROPHENYL)PIPERIDINO]PROPYL-CARBAMATE:
¹H NMR δ 6.91 (m, 2 H), 5.62 (b, 1 H), 4.31 (t, 2 H, J=5.4 Hz), 3.63 (m, 2 H), 3.39 (dt, 2 H, J= 2.1, 6.0 Hz), 3.40-2.70 (m, 7 H), 2.46 (t, 2 H, J=6.9 Hz), 2.10-1.60 (m, 4 H), 1.45 (s, 9 H).

10

3-[4-(3,4,5-TRIFLUOROPHENYL)PIPERIDINO]-1-PROPANAMINE: ¹H NMR δ 6.93 (m, 2 H), 4.30 (b, 1 H), 3.36 (b, 1 H), 3.06 (m, 2 H), 2.77 (m, 2 H), 2.43 (m, 2 H), 2.20-1.40 (m, 9 H).

15

1-BENZYL-4-(5-FLUORO-2-METHOXYPHENYL)-4-PIPERIDINOL: ¹H NMR δ 7.40-6.80 (m, 8 H), 3.94 and 3.85 (s, 3 H), 3.61 and 3.58 (s, 2 H), 2.80-1.90 (m, 8 H).

20

1-BENZYL-4-(5-FLUORO-2-METHOXYPHENYL)-1,2,3,6-TETRAHYDRO PYRIDINE: ¹H NMR δ 7.40-6.70 (m, 8 H), 5.84 (m, 1 H), 3.77 (s, 3 H), 3.64 (s, 2 H), 3.17 (m, 2 H), 2.68 (t, 2 H, J=5.7 Hz), 2.54 (m, 2 H).

25

4-(5-FLUORO-2-METHOXY)PHENYL PIPERIDINE: mp 254-258 °C; ¹H NMR δ 1.53-1.68 (m, 2H), 1.79 (d, J=11.7 Hz, 2H), 2.12 (dt, J=2.1 Hz, J=11.7 Hz, 1H), 2.77 (dt, J=1.8 Hz, J=12.3 Hz, 1H), 2.90-3.05 (m, 1H), 3.10-3.22 (m, 2H), 3.68 (s, 1H), 3.79 (s, 3H), 6.72-6.93 (m, 3H). Anal. Calcd. For C₁₂H₁₇NOFCl + 0.14 CH₂Cl₂: C, 56.60; H, 6.76; N, 5.44. Found: C, 56.60; H, 6.92; N, 5.28.

30

T E R T - B U T Y L
N-3-[4-(5-FLUORO-2-METHOXYPHENYL)PIPERIDINO]PROPYL-

CARBAMATE: ^1H NMR δ 6.90-6.70 (m, 3 H), 5.76 (b, 1 H), 3.80 (s, 3 H), 3.68 (m, 1 H), 3.40-2.90 (m, 4 H), 2.45 (t, 2 H, $J=6.6$ Hz), 2.20-1.60 (m, 9 H), 1.45 (s, 9 H).

5 3-[4-(5-FLUORO-2-METHOXYPHENYL)PIPERIDINO]-1-PROPANAMINE:
 ^1H NMR δ 7.00-6.80 (m, 3 H), 3.80 (s, 3 H), 3.05 (d, 2 H, $J=11.4$ Hz), 2.76 (t, 2 H, $J=6.9$ Hz), 2.43 (dd, 2 H, $J=7.8$ Hz), 2.05 (dt, 2 H, $J=2.4, 11.7$ Hz), 1.90-1.20 (m, 10 H).

10 T E R T - B U T Y L
4-(1-NAPHTHYL)-1,2,3,6-TETRAHYDRO-1-PYRIDINECARBOXYL-ATE:
 ^1H NMR δ 8.00-7.80 (m, 2 H), 7.76 (d, 1 H, $J=8.1$ Hz), 7.50-7.44 (m, 2 H), 7.42 (d, 1 H, $J=8.1$ Hz), 7.27 (d, 1 H, $J=8.1$ Hz), 5.76 (br, 1 H), 4.14 (m, 2 H), 4 or 3.29 (t, 2 H, $J=5.7$ Hz), 2.52 (br m, 2 H), 1.53 (s, 9H).

20 4-(1-NAPHTHYL)PIPERIDINE: HCl salt; mp 330-332 °C; ^1H NMR δ 1.66-1.70 (m, 2H), 2.20-2.26 (m, 2H), 2.30-2.43 (m, 2H), 2.72-2.84 (m, 1H), 3.15-3.26 (m, 2H), 7.42-7.56 (m, 4H), 7.78 (d, $J=8.1$ Hz, 1H), 7.90 (d, $J=8.1$ Hz, 1H), 8.04 (d, $J=8.1$ Hz, 1H). Anal. Calcd. For $\text{C}_{15}\text{H}_{18}\text{NOCl} + 0.20 \text{CH}_2\text{Cl}_2$: C, 68.96; H, 7.00; N, 5.29. Found: C, 68.64; H, 7.04; N, 5.24.

25 TERT-BUTYL N-3-[4-(1-NAPHTHYL)PIPERIDINO]PROPYLCARBAMATE:
 ^1H NMR δ 8.09 (d, 1 H, $J=8.4$ Hz), 7.86 (dd, 1 H, $J=1.8, 7.5$ Hz), 7.71 (dd, 1 H, $J=2.4, 6.9$ Hz), 7.60-7.30 (m, 4 H), 6.31 (br, 1 H), 5.75 (br, 1 H), 4.26 (t, 1 H, $J=5.4$ Hz), 3.40-3.00 (m, 6 H), 2.54 (t, 2 H, $J=6.9$ Hz), 2.24 (dt, 2 H, $J=3.0, 11.4$ Hz), 2.00-1.60 (m, 6 H), 1.45 (s, 9 H).

30 4-(3-METHYL-2-PYRIDYL)-4-PIPERIDINOL: ^1H NMR δ 8.21 (dd, 1 H, $J=1.2, 4.5$ Hz), 7.36 (dd, 1 H, $J=6.6, 7.8$ Hz), 7.02 (dd, 1 H, $J=4.8, 7.5$ Hz), 3.07 (dt, 2 H, $J=2.7, 12.3$ Hz),

2.89 (m, 2 H), 2.46 (s, 3 H), 2.22 (dt, 2 H, J=4.8, 12.3 Hz), 1.39 (dm, 2 H, J=12.3 Hz).

T E R T - B U T Y L
5 4-(3-METHYL-2-PYRIDYL)-1,2,3,6-TETRAHYDRO-1-PYRIDINE-CARBOXYLATE: ^1H NMR δ 8.16 (dd, 1 H, J=1.2, 3.3 Hz), 7.51 (dm, 1 H, J=7.5 Hz), 7.15 (dd, 1 H, J=4.8, 7.5 Hz), 5.73 (br, 1 H), 4.01 (m, 2 H), 3.59 (t, 2 H, J=5.7 Hz), 2.40 (m, 2 H), 1.44 (s, 9 H).

T E R T - B U T Y L
10 N-3-[4-(3-METHYL-2-PYRIDYL)PIPERIDINO]PROPYLCARBAMATE: ^1H NMR δ 8.37 (dd, 1 H, J=4.2, 4.8 Hz), 7.51 (dd, 1 H, J=7.2, 7.5 Hz), 7.20 (dd, 1 H, J=4.5, 7.5 Hz), 6.73 (br, 1 H),
15 3.26 (m, 4 H), 3.05 (d, 2 H, J=12.0 Hz), 2.80-2.40 (m, 4 H), 2.61 (s, 3 H), 1.82 (p, 2 H, J=6.3 Hz), 1.54 (d, 2 H, J= 12.0 Hz).

T E R T - B U T Y L
20 4-(3-METHOXYPHENYL)-1,2,3,6-TETRAHYDRO-1-PYRIDINECARBOXYLATE: ^1H NMR δ 7.23 (t, 1 H, J= 8.1 Hz), 6.96 (d, 1 H, J=7.5 Hz), 6.89 (d, 1 H, J=1.8 Hz), 6.80 (dd, 1 H, J=2.4, 8.1 Hz), 6.02 (br, 1 H), 4.20-4.00 (m, 3 H), 3.80 (s, 3 H), 3.62 (t, 2 H, J=5.7 Hz), 2.51 (br, 2 H), 1.49 (s, 9 H).

1-BENZYL-4-METHYL-PIPERIDIN-4-OL: Methylolithium (1.4 M in Et₂O, 54.0 mL) was added to a solution of 1-benzyl-4-piperidone (5.00 mL, 27.0 mmol) in anhydrous ether at -78 °C under argon. Stirring was continued at -78 °C for 1.5 hours. Ether (200 mL) and water (40 mL) were added, and the two phases were separated. The aqueous solution was extracted with Et₂O (3 x 50 mL). The combined organic solutions were dried over magnesium sulfate and

concentrated. The residue was chromatographed (EtOAc to EtOAc-MeOH 9/1), giving 4.81 g (87%) of the desired product as a colorless oil: ^1H NMR δ 1.21 (s, 3 H), 1.56 (dt, J = 13, 3 Hz, 2 H), 1.65 (td, J = 10, 4 Hz, 2 H), 2.35 (td, J = 10, 3 Hz, 2 H), 2.53 (m, 2 H), 7.24 (m, 1 H), 7.29 (m, 4 H); ^{13}C NMR δ 30.44, 39.37, 50.39, 63.80, 68.50, 127.56, 128.80, 129.80, 139.17.

1-BENZYL-4-METHYL-4-PHENYLPiperidine: 1-Benzyl-4-methyl-piperidin-4-ol (4.81 g, 23.4 mmol) was added to a suspension of AlCl_3 (15.62 g, 117 mmol) in benzene (100 mL) at room temperature under argon. The mixture was stirred at reflux for 24 hours, then cooled and poured cautiously into ice water (100 g of ice, 50 mL of water). The aqueous phase was adjusted to pH 11-12 by addition of 6 N aqueous NaOH at 0 °C, and extracted with EtOAc (3 x 100 mL). The combined organic solutions were dried over magnesium sulfate and concentrated. The residue was chromatographed (hexane- Et_2O 19/1 to 9/1, followed by hexane-EtOAc 3/1), giving the desired product (3.23 g, 52%) as a brown oil: ^1H NMR δ 1.25 (s, 3 H), 1.80 (m, 2 H), 2.17 (m, 2 H), 2.44 (m, 2 H), 2.55 (m, 2 H), 3.50 (s, 2 H), 7.25 (m, 1 H), 7.35 (m, 4 H); ^{13}C NMR δ 36.82, 37.65, 50.95, 54.93, 64.08, 126.19, 126.51, 127.59, 128.83, 128.95, 129.05, 129.89, 139.24.

4-METHYL-4-PHENYLPiperidine: Freshly prepared methanolic formic acid solution (4.4% by weight, 70 mL) was added to 1-benzyl-4-methyl-4-phenylpiperidine (3.23 g, 12.2 mmol). To the resulting solution was added 10% palladium on carbon (2.00 g). The mixture was stirred at room temperature for 24 hours. The solid was filtered out and washed with MeOH (30 mL), H_2O (15 mL), CH_2Cl_2 (30 mL) and MeOH (15 mL). The combined filtrate and washings were

concentrated, and the residue was dissolved in CH_2Cl_2 (50 mL) and H_2O (10 mL). The aqueous phase was adjusted to pH 11 by addition of 1 N aqueous NaOH. The organic phase was separated, dried over magnesium sulfate and concentrated.

5 The residual oil was purified by flash chromatography ($\text{CHCl}_3/\text{MeOH}/2\text{ N NH}_3$ in MeOH 100/4/0 to 100/20/10), giving 1-benzyl-4-methyl-4-phenylpiperidine (1.20 g) and 1.10 g (51%, 82% based on consumed starting material) of 4-methyl-4-phenylpiperidine: ^1H NMR δ 1.24 (s, 3 H), 1.71 (m, 2 H), 2.06 (m, 2 H), 2.82 (m, 3 H), 2.94 (m, 2 H), 7.19 (m, 1 H), 7.32 (m, 4 H); ^{13}C NMR δ 37.22, 38.54, 43.44, 47.74, 126.31, 127.43, 129.01, 149.73.

10 3-AMINOPROPYL-4-METHYL-4-PHENYLPiperidine: A solution of 4-methyl-4-phenylpiperidine (1.00 g, 5.70 mmol), 3-bromo-propylamine hydrobromide (1.87 g, 8.55 mmol) and potassium carbonate (1.97 g, 14.2 mmol) in refluxing dioxane (20 mL) was stirred for 36 hours. After removal of the solvent, water (50 mL) was added and the pH adjusted to 11-12 by the addition of 1 N aqueous NaOH. The mixture was extracted with CH_2Cl_2 (150 mL + 3 x 100 mL). The combined organic solutions were dried over magnesium sulfate and concentrated. The residue was purified by flash chromatography ($\text{CHCl}_3/\text{MeOH}/2\text{ N NH}_3$ in MeOH 100/20/10), giving the desired product as a colorless oil (241 mg, 18%): ^1H NMR δ 1.18 (s, 3 H), 1.61 (p, $J = 7$ Hz, 2 H), 1.75 (m, 2 H), 2.10 (m, 2 H), 2.33 (t, $J = 7$ Hz, 2 H), 2.40 (m, 2 H), 2.45 (m, 2 H), 2.72 (t, $J = 6$ Hz, 2 H), 3.02 (br s, 2 H), 7.14 (m, 1 H), 7.30 (m, 4 H); ^{13}C NMR δ 30.28, 36.78, 37.64, 41.51, 50.96, 57.51, 126.16, 126.40, 128.91, 149.20.

15 20 25 30

Preparation of 3-[4-(4-Fluorophenyl)piperidin-1-yl]propylamine

4-(4-FLUOROPHENYL) PIPERIDINE HYDROCHLORIDE: To a solution
of 4-(4-fluorophenyl)-1,2,3,6-tetrahydropyridine
hydrochloride (10 g) in methanol (200 mL) was added 10%
palladium on charcoal (0.5 g) and the mixture was
5 hydrogenated at 50 psi for 3 h. The catalyst was removed
by filtration and solvent was evaporated, leaving the
product (10.0 g) as a white powder, which was used in the
next step without purification. The product appeared to be
pure based on ^1H NMR and TLC analysis. ^1H NMR δ 1.95-2.03 (br
10 d, 2H), 2.14-2.29 (m, 2H), 2.70-2.80 (m, 1H), 2.91-3.07
(br q, 2H), 3.60-3.64 (br d, 2H), 6.96-7.03 (m, 2H),
7.19-7.22 (m, 2H), 9.60 (br s, 1H), 9.71 (br s, 1H).

4-(4-FLUOROPHENYL) PIPERIDINE: mp $^{\circ}\text{C}$; ^1H NMR δ 1.51-1.66 (m,
15 2H), 1.80 (d, $J=7.2$ Hz, 2H), 2.53-2.64 (m, 1H), 2.67-2.77
(m, 2H), 3.17 (d, $J=12.0$ Hz, 2H), 6.94-7.03 (m, 2H),
7.13-7.21 (m, 2H).
Anal. Calcd. For $\text{C}_{11}\text{H}_{14}\text{NF} + \text{C}_4\text{H}_4\text{O}_4$: C, 58.70; H, 5.83; N,
4.18.
20 Found: C, 58.72; H, 5.84; N, 3.98.

3-[4-(4-FLUOROPHENYL) PIPERIDIN-1-YL] PROPYLPHTHALIMIDE: A
mixture of 4-(4-fluorophenyl)piperidine hydrochloride
(5.08 g, 23.2 mmol), 3-bromopropylphthalimide (6.22 g,
25 23.2 mmol), and potassium carbonate (15 g) in DMF (100 mL)
was stirred at 95-100 $^{\circ}\text{C}$ for 12 h. About 80% of the
solvent was evaporated under reduced pressure. The
residue was diluted with ethyl acetate (200 mL) and washed
with brine (3 X 100 mL) and dried (Na_2SO_4). The solvent
30 was evaporated from the ethyl acetate solution and the
residue was purified by column chromatography (1/1
hexane-ethyl acetate to 100% ethyl acetate), giving crude
product (7.50 g, 88%). This crude product was crystallized
from isopropanol, giving a white crystalline solid (4.50

g, 1st crop). This material was used in the next step. Concentration of the mother liquor and cooling gave the second crop of desired product (1.0 g). ^1H NMR δ 1.43-1.52 (m, 2H), 1.67-1.75 (m, 2H), 1.80-1.96 (m, 4H), 2.33-2.46 (m, 3H), 2.94-2.99 (br d, 2H), 3.78 (t, J=7 Hz, 2H), 6.90-7.04 (m, 4H), 7.70-7.74 (m, 2H), 7.84-7.87 (m, 2H).

3 - [4 - (4 - FLUOROPHENYL) PIPERIDIN-1-YL] PROPYLAMINE:
Hydrazine (4 mL) was added to a solution of
10 3 - [4 - (4-fluorophenyl)piperidin- 1-yl]propylphthalimide
4.50 g, 12.3 mmol) in methanol (200 mL), and the mixture
was stirred at reflux for 8 h. The solution was cooled to
room temperature, and the resulting white solid which
formed was filtered and washed with methanol (20 mL). The
15 solvent was evaporated from the filtrate and residue was
dried under vacuum for 4 h. The crude product was
dissolved in 50 mL of chloroform, stirred for 1 h, and
filtered. The white solid was washed with additional
chloroform (20 mL), the solvent was evaporated from the
20 combined filtrates to leave the crude product as an oil.
The oil was purified by column chromatography
(dichloromethane / methanol / 2 M ammonia in methanol,
10/3/1), giving the desired product (2.70 g, 93%). ^1H NMR
 δ 1.60-1.83 (m, 6H), 1.96-2.07 (m, 4H), 2.40-2.55 (m, 3H),
25 2.70-2.85 (br t, 2H), 3.03-3.07 (br d, 2H), 6.93-7.00 (m,
2H), 7.14-7.20 (m, 2H).

4 - (4 - METHYL-4 - (3 ,5 -DIMETHYLPHENYL) PIPERIDINE: hygroscopic;
 ^1H NMR δ 1.20 (s, 3H), 1.74-1.80 (m, 2H), 2.08-2.16 (m, 2H),
30 2.30 (s, 6H), 2.50-2.56 (m, 2H), 2.64-2.68 (m, 2H),
2.97-3.04 (m, 1H), 6.87 (s, 1H), 6.94 (s, 2H).

BENZYL 4 - { [(TERT-BUTOXYCARBONYL) AMINO] METHYL }

CYCLOHEXYLCARBAMATE:

Oxalyl chloride (1.1 equivalents) was added dropwise to a mixture of 4-[(tert-butoxycarbonyl)-amino]methyl cyclohexanecarboxylic acid (1 equivalent, Maybridge) in toluene. The reaction mixture was stirred at room temperature for 2-6 h. The solvent was removed in vacuo, the residue was dissolved in acetone and the resulting mixture was added dropwise to an aqueous solution of sodium azide (1.2 equivalents) at a rate such as to maintain a temperature of 10-15 °C. After the completion of the reaction, the reaction mixture was extracted with ethyl acetate, the combined extracts were dried and concentrated in vacuo. The residue was dissolved in acetone and added slowly to warm (60 °C) benzene. After the completion of the reaction, benzyl alcohol was added to the reaction mixture, stirred for 2 days and the desired product was isolated (For Typical References, See: G. Schroeter Ber. 1909, 42, 3356; and Allen, C.F.H.; Bell, A. Org. Syn. Coll. Vol. 3 (1955) 846.).

A solution of benzyl 4-[(tert-butoxycarbonyl)amino]methyl-cyclohexylcarbamate in MeOH containing 10% Pd/C was hydrogenated at 50 psi overnight. The reaction mixture was filtered through Celite 545 and the Celite 545 was washed with methanol. The combined methanol extracts were concentrated in vacuo, giving *trans*-tert-butyl 4-aminocyclohexylmethylcarbamate (95 %).

9 H - 9 - F L U O R E N Y L M E T H Y L
N-[4-(AMINOMETHYL)CYCLOHEXYL]CARBAMATE: : ¹H NMR δ 8.02 (br, 1 H), 7.33 (m, 5 H), 5.07 (s, 2 H), 3.71 (s, 1 H), 3.40 (br m, 1 H), 2.80 (br m, 2 H), 1.94 (ABq, 4 H), 1.68 (br, 1 H), 1.30-1.00 (m, 5 H).

N1 - [4 - (AMINOMETHYL) CYCLOHEXYL] -1-NAPHTHAMIDE: HCl in dioxane (10 mL, 4 N) was added to a solution of *tert*-butyl[4-(1-naphthoyl-amino)cyclohexyl]methylcarbamate (0.350 g) in dichloromethane (20 mL), stirred overnight, concentrated *in vacuo*, giving the desired product: ^1H NMR δ 8.24 (dd, 1 H, $J=1.2, 8.7$ Hz), 7.85 (dt, 2 H, $J=2.7, 9.7$ Hz), 7.60-7.30 (m, 4 H), 5.98 (m, 1 H), 4.02 (m, 1 H), 3.80-3.40 (m, 4 H), 2.53 (d, 2 H, $J=6.0$ Hz), 2.02 (ABq, 4 H), 1.41-1.90 (m, 4 H).

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TERT-BUTYL N- (4 - [(1-NAPHTHYLCARBONYL)AMINO] CYCLOHEXYLMETHYL) -CARBAMATE: A mixture of 1-naphthoic acid (1.00 mmol, 0.172 g), DMAP (2.00 mmol, 0.250 g) and ECD (0.383 g, 2.00 mmol) in dry dichloromethane (20 mL) was stirred at room temperature for 0.5 h followed by the addition of *tert*-butyl(4-amino)cyclohexyl)methyl-carbamate amine (1.09 mmol, 0.250 g). The reaction mixture was stirred at room temperature overnight and purified by flash chromatography, giving the desired product as a white solid (0.160 g): ^1H NMR δ 8.29 (dd, 1 H, $J=1.8, 9.1$ Hz), 7.89 (m, 2 H), 7.60-7.40 (m, 4 H), 5.85 (br d, 1 H, $J=6.3$ Hz), 4.65 (m, 1 H), 4.04 (m, 1 H), 3.02 (t, 1 H, $J=6.3$ Hz), 2.05 (ABq, 4 H), 1.62 (m, 2 H), 1.46 (s, 9 H), 1.40-1.10 (m, 4 H).

25

4-ACETYL-1-(3-AMINOPROPYL)-4-PHENYLPiperidine: A solution of 4-Acetyl-4-phenylpiperidine (7, 1.53 g, 7.50 mmol), 3-bromo-propylamine hydrobromide (1.64 g, 7.50 mmol) and potassium carbonate (1.24 g, 9.00 mmol) was stirred in refluxing 1,4-dioxane (50 mL) for 12 h. After removal of dioxane, water (50 mL) was added and the pH was adjusted to 11-12 by addition of 1 N aqueous NaOH. The mixture was extracted with CH_2Cl_2 (100 mL + 3 x 50 mL). The combined organic solutions were dried over magnesium sulfate and

concentrated. The residue was purified by flash chromatography (EtOAc-MeOH-Et₃N 100/40/20), giving the desired product as a colorless oil (780 mg, 40%): ¹H NMR δ 1.56 (p, J = 7 Hz, 2 H), 1.84 (s, 3 H), 1.98 (m, 2 H),
5 2.15 (br t, J = 12 Hz, 2 H), 2.29 (t, J = 7 Hz, 2 H), 2.41 (br d, J = 12 Hz, 2 H), 2.66 (t, J = 7 Hz, 4 H), 7.18 - 7.30 (m, 5 H); ¹³C NMR δ 26.28, 31.11, 33.43, 41.47, 51.62, 55.31, 57.19, 77.32, 77.74, 78.17, 126.95, 127.69, 129.44, 142.25, 210.15.

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For the preparation of benzo-4',5' [H]furanpiperidine refer to W.E.Parham et al, *J. Org. Chem.* (1976) 41, 2268.

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TERT-BUTOXY{[3-(BENZO-4',5' [H]FURANPIPERIDIN-1-YL)PROPYL]AMINO}METHANOL: To a stirred solution of the N-[4-(benzo-4',5' [H]furanpiperidine (0.566 g, 3.27 mmol) in dioxane (20 mL), N-(tert-butoxycarbonyl)-3-bromopropylamine (0.772 g, 3.27 mmol) and potassium carbonate (0.904 g, 6.54 mmol) were added and the solution was refluxed for 24 h. The reaction mixture was cooled to room temperature, concentrated and partitioned between chloroform (40 mL) and water (5 mL). The organic layer was dried over sodium sulfate, filtered and concentrated. The crude product was purified by column chromatography (ethyl acetate/methanol, 4.5/0.5), giving the desired product as a colorless oil (0.856 g, 79 %); ¹H NMR (1.45 (s, 9 H), 1.63-2.04 (m, 6 H), 2.33-2.52 (m, 4 H), 2.87 (d, J=11.0 Hz, 2 H), 3.2 (br s, 2 H), 5.07 (s, 2 H), 5.6 (br s, 1 H), 7.13-7.28 (m, 4 H).

3-(4-METHYL-4-PHENYL-1-PIPERDINYLPROPYLAMINE: Trifluoroacetic acid (1 mL) was added to tert-butoxy{[3-(4-methyl-4-phenyl-1-piperdinyl)propyl]-

amino}methanol (0.500 g, 1.51 mmol) in dichloromethane (5 mL) and the solution was stirred at room temperature for 1 h. The solution was concentrated, neutralized with 10 % KOH solution and extracted with dichloromethane (25 mL).
5 The organic layer was dried over sodium sulfate, filtered and concentrated, giving 0.340 g (98%) of 3-(4-methyl-4-phenyl-1-piperdanyl)propylamine which was used without further purification in the subsequent step.

10 Procedures for the Reaction of the Amine Side Chains with the p-Nitrophenylcarbamate Intermediates:

General Procedure:

An equimolar solution of an amine side chain such as 3-(4-methyl-4-phenyl-1-piperdanyl)propylamine and a p-nitrophenylcarbamate intermediate such as 5-methoxy carbonyl-4-methoxy methyl-1,2,3,6-tetrahydro-2-oxo-6-(3,4-difluorophenyl)-1-[(4-nitrophenyloxy)carbonyl]pyrimidine and 1-2 equivalents of a base such as diisopropylethylamine in dichloromethane were stirred at room temperature overnight. The reaction mixture was concentrated and purified by flash chromatography, giving the desired product. In case of 2-methoxy intermediates, conversion to the oxo derivatives was accomplished by treatment of the 2-methoxy product with HCl in dioxane.
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2-OXO-3-{SPIRO[1H-INDANE-1,4'-PIPERIDINE]} PROPYLAMINE (0.0319 g, 0.123 mmol) was added to (\pm)-6-(3,4-difluorophenyl)-1,6-dihydro-2-methoxy-5-methoxycarbonyl-4-ethyl-1-(4-nitrophenoxy)carbonyl-pyrimidine (0.052 g, 0.112 mmol) in dry dichloromethane (10 mL) and the solution was stirred at room temperature for 24 h. The reaction mixture was stirred for another 1 h after addition of 6 N HCl (2 mL). After neutralization with
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aqueous 10% KOH solution, the reaction mixture was extracted into dichloromethane (3×10 mL). The organic layer was dried over sodium sulfate, filtered and concentrated. The crude product was purified by flash chromatography (EtOAc/ MeOH, 4.5/0.5), giving of the desired product (0.040 g) as a syrup.

1 N HCl in ether (5 mL) was added to the free base (0.040 g, 0.072 mmol) in dichloromethane (4 mL) and the solution was concentrated under reduced pressure. The crude product was recrystallized from ether, giving the desired compound (0.042 g, 99 %) as a pale yellow solid; mp 178-182 °C; Anal. Calcd. for $C_{29}H_{34}F_2N_4O_5Cl_2 + 0.6 H_2O$: C, 57.87; H, 5.73, N 9.31. Found: C, 58.11; H 5.90; N 8.95.

General Procedure for the reaction of the piperidines and piperazines with 1-(3-bromo-propylcarbamoyl)-6-(3,4-difluoro-phenyl)-4-methyl-2-oxo-1,6-dihydro-pyrimidine-5-carboxylic acid methyl ester:

The amine (0.15 mmol) was added to a solution of 1 - (3 - b r o m o - propylcarbamoyl) - 6 - (3 , 4 - difluorophenyl) - 4 - methyl - 2 - oxo - 1 , 6 - di - hydro - pyrimidine - 5 - carboxylic acid methyl ester (43.0 mg, 0.100 mmol) in anhydrous acetone (10 mL), followed by NaHCO₃ (41 mg, 0.3 mmol) and KI (16 mg, 0.1 mmol). The resulting suspension was heated to reflux for 10 h and then cooled to room temperature. The solvent was removed *in vacuo* and the residue was purified by flash column chromatography (EtOAc, followed by EtOAc/MeOH, 9/1). The product was then dissolved in 2 mL of chloroform, acetone or EtOAc and HCl in Et₂O (1 M, 0.5 mL) was added at room temperature. The solvent was removed *in vacuo*, giving the desired compound as an HCl salt.

Example 1

(
)-1,2,3,6-TETRAHYDRO-1-{N-[4-(3,-ACETAMIDO)-PHENYL-PIPER-
IDIN-1-YL]PROPYL}CARBOXAMIDO-4-METHOXYMETHYL-
6-(3,4-DIFLUORO-PHENYL)-2-OXOPYRIMIDINE-5-CARBOXYLIC ACID
5
METHYL ESTER: ESMS, 612.25 (M+1); ^1H NMR δ 1.76-1.87 (m,
6H), 2.03-2.13 (m, 2H), 2.18 (s, 3H), 2.49 (t, J=6.9 Hz,
3H), 3.10 (d, J=11.1 Hz, 2H), 3.30-3.42 (m, 2H), 3.45 (s,
3H), 3.71 (s, 3H), 4.68 (s, 2H), 6.68 (s, 1H), 6.96 (d,
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J=7.5 Hz, 1H), 7.04-7.11 (m, 2H), 7.16-7.26 (m, 2H), 7.34
(d, J=6.3 Hz, 1H), 7.45 (s, 1H), 7.94 (s, 1H), 8.98 (t,
J=5.4 Hz, 1H).

15 **Example 2**

M	E	T	H	Y	L
3 - [(3-4 - [3 - (ACETYLAMINO) PHENYL] -1,2,3,6-TETRAHYDRO-1-PYR- IDINYLPROPYL) AMINO] CARBONYL-4-(3,4-DIFLUOROPHENYL)-6-(ME- THOXY-METHYL)-2-OXO-1,2,3,4-TETRAHYDRO-5-PYRIMIDINE- 20 CARBOXYLATE: ^1H NMR δ 8.90 (t, 1 H, J=3.6 Hz), 7.75 (s, 1 H), 7.50-7.00 (m, 8 H), 6.68 (s, 1 H), 6.03 (br s, 1 H), 4.67 (s, 2 H), 3.71 (s, 3 H), 3.47 (s, 3 H), 3.38 (ABm, 2 H), 3.16 (m, 2 H), 2.71 (t, 2 H, J =5.4 Hz), 2.56 (m, 4 H), 2.35-1.90 (br, 2 H), 2.17 (s, 3 H), 1.82 (p, 2 H, 25 J=7.2 Hz); ESMS, 612.25 (M+1).					

Example 3

(1)-1,2,3,6-TETRAHYDRO-1-{N-[3-(4-O-ACETYL)-4-PHENYLPIPE-
RIDIN-1-YL]PROPYL}CARBOXAMIDO-5-METHOXYCARBONYL-
4-METHOXYMETHYL-6-(3,4-DIFLUOROPHENYL)-2-OXOPYRIMIDINE:
30
4-Acetyl-1-(3-aminopropyl)-4-phenylpiperidine (190 mg,
0.687 mmol) was added to a stirring solution of
5-methoxycarbonyl-4-methoxymethyl-1,2,3,6-tetra-
hydro-2-oxo-6-(3,4-difluorophenyl)-1-[(4-nitrophenyloxy)

carbon-yl]pyrimidine (281 mg, 0.573 mmol) in dry dichloromethane (3 mL) and THF (4 mL). The reaction mixture was stirred at room temperature for 12 h. The reaction mixture was quenched with aqueous 6 N HCl. The reaction mixture was concentrated to a small volume, partitioned between dichloromethane and water (100 mL each), the mixture was adjusted to pH 8 by addition of Na₂CO₃, the layers were separated, and the aqueous layer was extracted with dichloromethane (3 x 30 mL). The combined organic extracts were dried (Na₂SO₄) and the product was chromatographed, giving the desired product. The HCl salt was prepared by the addition of 1 N HCl in ether to a solution of the product in CH₂Cl₂. The precipitated salt was filtered, washed with ether and dried in vacuo, giving (1)-1,2,3,6-tetrahydro-1-{N-[3-(4-O-acetyl)-4-phenylpiperidin-1-yl]propyl}carboxamido-5-methoxycarbonyl-4-methoxymethyl-6-(3,4-difluorophenyl)-2-oxopyrimidine (170 mg, 47%) as the hydrochloride salt: (C₃₁H₃₆N₄F₂O₇ + HCl + 0.6 CH₂Cl₂); mp 82-84 °C.

Example 4

Benzyl ester precursor to the product of Example 4:

(+)-1,2,3,6-TETRAHYDRO-1-{N-[4-(BENZO-4',5' (H) FURAN) PIPE RIDIN-1-YL]PROPYL}-CARBOXAMIDO-4-ETHYL-6-(3,4-DIFLUOROPHENYL)-2-OXO-PYRIMIDINE-5-CARBOXYLIC ACID PHENYLMETHYL ESTER: ¹H NMR δ 7.60-7.00 (m, 12 H), 6.85 (br, 1 H), 6.62 (s, 1 H), 5.10 (ABq, 2 H), 5.67 (s, 2 H), 4.03 (br, 1 H), 4.01 (s, 3 H), 3.40 (apparent q, 2 H, J=6.8 Hz), 3.20-1.60 (m, 12 H), 2.86 (q, 2 H, J=2.5 Hz), 1.19 (t, 3 H, J=7.5 Hz).

(+)-1,2,3,6-TETRAHYDRO-1-{N-[4-(BENZO-4',5' (H) FURAN) PIPE R I D I N - 1 - Y L] P R O P Y L } -

CARBOXAMIDO-4-ETHYL-6-(3,4-DIFLUOROPHENYL)-

2-OXO-PYRIMIDINE-5-CARBOXYLIC ACID HYDROCHLORIDE: ^1H NMR
δ 8.95 (br s, 1 H), 8.22 (br s, 1 H), 7.40-6.95 (m, 7 H),
6.95 (s, 1 H), 6.63 (s, 1 H), 5.10-4.95 (m, 2 H),
5 3.40-3.20 (m, 4 H), 3.10-2.80 (m, 4 H), 2.55-2.20 (m, 1 H),
H, 2.15 (m, 1 H), 1.85 (m, 2 H), 1.55-1.30 (m, 4 H), 1.20
(t, 3 H, $J=7.6$ Hz); Anal. Calc. For $\text{C}_{29}\text{H}_{32}\text{N}_4\text{O}_5\text{F}_2 + \text{HCl} + 1.5\text{H}_2\text{O}$: C, 56.36; H, 5.87; N, 8.06. Found: C, 56.72; H, 6.11;
N, 7.61.

10

Example 5

1,2,3,4-TETRAHYDRO-1-OXO-2-NAPHTHACETIC ACID METHYL ESTER:
Under argon, α -tetralone (5.00 g, 34.2 mmol) in dry THF
(300 mL) was treated with LDA in THF (2 M, 18.8 mL) at -78
15 °C. The solution was stirred at -78 °C for 1 h. Methyl
bromoacetate (15.7 g, 0.103 mole) was then added to the
solution, the mixture was stirred overnight and allowed to
warm to room temperature. The solvent was evaporated and
the residue was dissolved into CHCl_3 (300 mL), washed with
water and saturated brine, and then dried over Na_2SO_4 .
20 After filtration and removal of solvent, the residue was
vacuum distilled. The product, a colorless oil (7.21 g,
96.5%) was collected at 180 °C/1 mm Hg; ^1H NMR (400 MHz) δ
1.98 (m, 1H), 2.25 (m, 1H), 2.44 (m, 1H), 2.90-3.20 (m,
25 4H), 3.73 (s, 3H), 7.10-8.10 (m, 4H); EI mass spectrum M+
at m/z 218.

1-HYDROXY-2-(2-HYDROXYETHYL)-1,2,3,4-TETRAHYDRONAPHTHALENE:
A solution of 1,2,3,4-tetrahydro-1-oxo-naphthacetic
30 acid methyl ester (6.15 g, 28.2 mmol) in THF (150 mL) was
treated with LiAlH_4 (2.82 g, 70.5 mmol) and then the
reaction mixture was heated at reflux temperature for 5 h.
The suspension was cooled to 0 °C and quenched by addition
of solid $\text{Na}_2\text{SO}_4 \cdot 10\text{H}_2\text{O}$. The mixture was stirred at room

temperature for 4 hrs. The solid was removed by filtration and concentration of the filtrate *in vacuo* gave a yellow oil (5.33 g, 98.3%); ¹H NMR indicated the formation of an isomeric mixture. EI mass spectrum M⁺ at m/z 192. The mixture was directly used in next reaction without further purification.

2 - (2-HYDROXYETHYL) -1,2,3,4-TETRAHYDRO-1-OXO-NAPHTHALENE:
A solution of isomeric mixture of
10 1 - hydroxyl - 2 - (2 - hydroxyethyl) -
1,2,3,4-tetrahydronaphthalene (3.00 g, 15.6 mmol) in CH₂Cl₂
(100 mL) was treated with MnO₂ (20.4 g, 0.234 mole). The suspension was stirred at room temperature for 16 h and the solids were removed by filtration. Concentration of
15 the filtrate *in vacuo* gave a brown oil, which was further purified by flash chromatography (MeOH/ CHCl₃, 5/95), giving a yellow oil (2.00 g, 67.4%): ¹H NMR δ 1.76 (m, 1H), 1.98 (m, 1H), 2.21 (m, 2H), 2.57 (br, 1H), 2.70 (m, 2H), 3.20 (m, 2H), 3.81 (m, 2H), 7.00-8.20 (m, 4H); CI mass spectrum (M+1)⁺ at m/z 191.

2 - (2-BROMOETHYL) -1,2,3,4-TETRAHYDRO-1-OXONAPHTHALENE: A solution of 2-(2-hydroxethyl)-1,2,3,4-tetrahydro-
1-oxo-naphthalene (2.00 g, 10.5 mmol) in CH₂Cl₂ (100 mL)
25 was treated with PBr₃ (948 mg, 3.50 mmol) at 0 °C. The mixture was stirred at room temperature for 72 h and then poured onto 100 g of ice. The organic layer was separated, washed with aqueous 10% K₂CO₃ solution, H₂O, saturated NaCl and dried over Na₂SO₄. After filtration and removal of the solvent, the residue was purified by chromatography (EtOAc/hexane, 1/10), giving a yellow oil (1.18 g, 44.4%); ¹H NMR δ 1.49 (m, 2 H), 2.24 (m, 1H), 2.60 (m, 1H), 2.75 (m, 1H), 3.03 (m, 2H), 3.64 (m, 2H), 7.10-8.10 (m, 4H); EIMS M⁺ m/z 223, M/M+2=1:1.

2- [2- (4-BENZAMINO-1-PIPERIDYL) ETHYL] -1,2,3,4-TETRAHYDRO-
 1-OXO- NAPHTHALENE: A mixture of 2-(2-bromoethyl)-1,2,3,4-
 tetrahydro-1-oxonaphthalene (1.18 g, 4.66 mmol),
 4-benzamidopiperidine (952 mg, 4.66 mmol) and K₂CO₃ (1.29
 g, 9.32 mmol) in acetone (200 mL) was stirred at room
 temperature for 48 h. The solids were removed by
 filtration. Concentration of filtrate *in vacuo* gave a
 yellow solid which was purified by chromatography (MeOH:
 CHCl₃, 5/95). The product was recrystallized from an
 EtOAc/hexane mixture, giving a white powder (268 mg,
 15.3%); mp 158-159 °C; ¹H NMR δ 1.53 (m, 2H), 1.67 (m, 1H),
 1.91 (m, 1H), 2.02 (m, 2H), 2.21 (m, 4H), 2.50 (m, 3H),
 2.95 (m, 4H), 4.01 (m, 1H), 5.95 (d, J=8.0 Hz, 1H),
 7.20-8.10 (m, 9H); CI MS (M+1) +m/z 377; Anal. Calcd for
 C₂₄H₂₈N₂O₂: C, 76.55; H, 7.51; N, 7.44. Found: C, 76.28; H,
 7.46; N, 7.37.

Example 6

M	E	T	H	Y	L	
20	4- (2 , 1 , 3 - BENZOXADIAZOL - 5 - YL) - 3 - [(1 - [4 - (DIBUTYLAMINO) - BENZYL] - 4 - PIPERIDYL METHYL) AMINO] CARBONYL - 6 - METHYL - 2 - OXO - 1 , 2 , 3 , 4 - TETRAHYDRO - 5 - PYRIMIDINE CARBOXYLATE: ¹ H NMR δ 7.72 (dd, 1 H, J=0.6, 9.6 Hz), 7.70-7.50 (m, 2 H), 7.11 (d, 2 H, J=8.7 Hz), 6.59 (d, 2 H, J=8.7 Hz), 5.90 (s, 1 H), 3.94 (s, 3 H), 3.63 (s, 2h), 3.24 (t, 4 H, J=7.8 Hz), 2.80 (m, 2 H), 2.49 (d, 2 H, J=6.3 Hz), 2.38 (s, 3 H), 2.90-1.00 (m, 5 H), 1.54 (p, 4 H, J= 7.8 Hz), 1.35 (sextet, 4 H, J=7.8 Hz), 0.94 (t, 6 H, J=7.8 Hz).					21
25						22

30 Example 7

(+)-1,2,3,6-TETRAHYDRO-1-{N-[4-(N'-ETHYL)-N-BENZIMIDAZOLYL]PIPERIDIN-1-YL}PROPYL}CARBOXAMIDO-4-METHYL-6-(3,4-DIFLUOROPHENYL)-2-OXOPYRIMIDINE HYDROCHLORIDE: ^1H NMR δ 8.95 (t,

1 H, J=3.6 Hz), 7.61 (b, 1 H), 7.60-6.95 (m, 7 H), 6.69
5 (s, 1 H), 4.36 (m, 1 H), 3.94 (q, 2 H, J=7.2 Hz), 3.72 (s,
3 H), 3.42 (ABm, 4 H), 3.30 (m, 2 H, 4.76 (m, 4 H), 2.43
J=7.2 Hz).

Example 8

6-(BENZOFURAZAN-5-YL)-1,2,3,6-TETRAHYDRO-5-METHOXYSUBSTITUTED
YL-4-METHYL-2-OXO-1-{N-[3-(4-PHENYLPiperidin-1-YL)
10 PROPYL]}CARBOXAMIDO-PYRIMIDINE: A solution of 6-(benzo-
f u r a z a n - 5 - y l) - 1 , 6 - d i h y d r o - 2 -
methoxy-5-methoxycarbonyl-4-methyl-1-{N-[3-(4-phenylpiperidin-1-
yl)propyl]}carboxamidopyrimidine in MeOH was
treated with 6 N HCl at 0 °C. The solution was stirred at
15 room temperature for 2 h and the MeOH was removed in
vacuo. 6-(Benzofurazan-5-yl)-
1,2,3,6-tetrahydro-5-methoxycarbonyl
- 4 - meth y l - 2 - o x o - 1 - { N - [3 - (4 -
phenylpiperidin-1-yl)propyl]}carboxamidopyrimidine
20 hydrochloride was obtained as a white powder: mp 134-137
°C.

Example 9

4-(3-METHOXY)-PHENYL PIPERIDINE: HCl salt; mp 150-154 °C;
25 ¹H NMR δ 2.04 (s, br, 2H), 2.25 (s, br, 2H), 2.80 (s, br,
1H), 3.09 (s, br, 2H), 3.66 (s, 2H), 3.78 (s, 3H), 6.79
(s, br, 3H), 7.23 (s, 1H), 9.41 (s, br, 1H). Anal. Calcd.
For C₁₂H₁₈NOCl + 0.30 CH₂Cl₂: C, 58.34; H, 7.40; N, 5.53.
Found: C, 58.30; H, 7.71; N, 5.35.

30 (+)-1,2,3,6-TETRAHYDRO-1-N-[4-(3-METHOXY)-PHENYL]-PIPERI-
DIN-1-YL] -PROPYL-CARBOXAMIDO-4-METHOXYMETHYL-6-(3,4-DIFLUOROPHENYL)-
2-OXOPYRIMIDINE-5-CARBOXYLIC ACID METHYL ESTER: mp 80-84 °C; [α]_D = +94.7, (c = 0.25, MeOH); ¹H NMR

δ 1.74-1.84 (m, 6H), 1.99-2.09 (m, 2H), 2.38-2.51 (m, 3H),
3.03 (d, J=11.1 Hz, 2H), 3.24-3.43 (m, 2H), 3.48 (s, 3H),
3.71 (s, 3H), 3.80 (s, 3H), 4.72 (s, 2H), 6.68 (s, 1H),
6.72-6.84 (m, 3H), 7.05-7.11 (m, 2H), 7.15-7.27 (m, 2H),
5 7.72 (s, 1H), 8.84 (t, J=5.4 Hz, 1H). Anal. Calcd. For
 $C_{30}H_{37}N_4O_6F_2Cl$: C, 57.8; H, 6.0; N, 9.0. Found: C, 57.61;
H, 6.57; N, 6.97.

Example 10

10 (+)-1,2,3,6-TETRAHYDRO-1-{N-[4-(3,-ACETAMIDO)-PHENYL-PIPERIDIN-1-YL]PROPYL}CARBOXAMIDO-4-METHOXYMETHYL-6-(3,4-DI
FLUORO-PHENYL)-2-OXOPYRIMIDINE-5-CARBOXYLIC ACID METHYL
ESTER: mp 135-138 °C; $[\alpha]_D = +105.5$, (c = 0.11, MeOH);
ESMS, 614.25 (M+1); 1H NMR δ 1.76-1.87 (m, 6H), 2.03-2.13
15 (m, 2H), 2.18 (s, 3H), 2.49 (t, J=6.9 Hz, 3H), 3.10 (d,
J=11.1 Hz, 2H), 3.30-3.42 (m, 2H), 3.46 (s, 3H), 3.71 (s,
3H), 4.68 (s, 2H), 6.68 (s, 1H), 6.96 (d, J=7.5 Hz, 1H),
7.04-7.11 (m, 2H), 7.16-7.26 (m, 2H), 7.34 (d, J=6.3 Hz,
1H), 7.45 (s, 1H), 7.94 (s, 1H), 8.97 (t, J=5.4 Hz, 1H);
20 ESMS, M+1 614.25

The compound of Example 10 may also be prepared via
hydrogenation of the compound of example 2 (H₂ balloon
method, methanol, Pd/C, overnight). A synthetic path
analogous to the latter route (Scheme 11) was used in the
preparation of the tritiated analog, which in turn, was
25 used as a radioligand in the MCH pharmacological assays.

Example 11

3-(4-PHENYLPiperidin-1-yl)PROPIONITRILE: Acrylonitrile
30 (3.1 mL, 44 mmol, 2.5 eq) was added to a solution of
4-phenylpiperidine (3.00 g, 18.0 mmol) in EtOH (40 mL) and
the mixture was stirred at room temperature for 1.5 h.
The volatiles were removed, giving 3.80 g of the desired
product (brown oil, 99%).

3 - (4-PHENYLPiperidin-1-YL) PROPYLAMINE: A solution of BH₃ in THF (1.0 M, 83.0 mL, 83.0 mmol, 3.5 eq) was added to a stirring solution of 3-(4-phenylpiperidin-1-yl)-propionitrile (5.10 g, 24.0 mmol) in anhydrous THF (20 mL) under argon at room temperature. The mixture was heated at reflux temperature for 4.5 hours and then cooled to room temperature. Aqueous 6 N HCl (130 mL) was added and stirring was continued for 2 hours at 50-70 °C. The mixture was basified to pH 9 by addition of aqueous 6 N NaOH and extracted with EtOAc (100 mL) and CH₂Cl₂ (3 x 100 mL). The combined organic extracts were dried over magnesium sulfate and concentrated. The residue was dissolved in CH₂Cl₂ (20 mL) and treated with HCl in ether (1.0 M, 50 mL). The solvents were removed, ether (250 mL) was added, the mixture was filtered, and the filter cake was washed with ether. Water (60 mL) was added to the resulting white solid, 1 N NaOH was added until pH 10-11 was reached, and then the aqueous phase was extracted with CH₂Cl₂ (3 X 50 mL). The combined extracts were dried over magnesium sulfate and the solvents were evaporated, giving the desired product (4.50 g, 87%).

6 - (3,4-DIFLUOROPHENYL) - 1,2,3,6 - TETRAHYDRO - 5 - METHOXCARBO
N Y L - - 4 -
25 METHYL - 2 - OXO - 1 - { N - [3 - (4 - PHENYLPiperidin - 1 - YL) PROPYL] }
CARBOXAMIDO - PYRIMIDINE: A solution of 6 - (3,4-difluorophenyl) - 1,6-dihydro - 2-methoxy - 5-methoxy carbonyl - 4 - methyl - 1 - { N - [3 - (4 - phenyl - piperidin - 1 - yl) propyl] } carboxamido pyrimidine (100 mg, 0.185 mmol, mp = 43-45 °C) in MeOH (5 mL) was treated with aqueous 6 N HCl (1.5 mL) at 0 °C. The solution was stirred at room temperature for 2 hrs and MeOH was removed in vacuo. 6 - (3,4-Difluorophenyl) - 1,2,3,6-tetrahydro - 5 - methoxycarbonyl - 4 - methyl - 2 - oxo - 1 - { N - [3 - (4 - phenylpiperidin - 1 - yl) propyl] } carboxamido pyrimidine

hydrochloride was obtained as a white powder (89 mg, 86%).
mp 133-136 °C.

Example 12

- 5 3-{(3,4,5-TRIFLUOROPHENYL)METHYLENE}-2,4-PENTANEDIONE: A stirring mixture of 3,4,5-trifluorobenzaldehyde (4.2 g, 26.2 mmol), 2,4-pentanedione (2.62 g, 26.2 mmol), piperidine (0.430 g, 5 mmol) in benzene (150 mL) was heated at reflux temperature (equipped with a Dean-Stark trap) for 8 h. The benzene was evaporated, the yellow oily residue, 2-{(3,4,5-trifluorophenyl)-methylene}-2,4-pentanedione, was used in the next step without further purification.
- 10 15 6-(3,4,5-TRIFLUOROPHENYL)-1,6-DIHYDRO-2-METHOXY-5-ACETYL-4-METHYLPYRIMIDINE: A stirring mixture of 2-{(3,4,5-trifluoro-phenyl)methylene}-2,4-pentanedione (26.2 mmol), O-methylisourea hydrogen sulfate (3.22 g, 39.3 mmol), and NaHCO₃ (6.60 g, 78.6 mmol) in EtOH (400 mL) was heated at 95-100 °C for 6 h. The mixture was filtered, the solid residue was washed with ethanol (100 mL). The solvent was evaporated from the combined filtrates and the crude product was purified by flash column chromatography (EtOAc/hexane, 9/1 to 4/1), giving the desired product as an oil (2.80 g, 36%).
- 20 25 6-(3,4,5-TRIFLUOROPHENYL)-1,6-DIHYDRO-2-METHOXY-5-ACETYL-4-METHYL-1-[(4-NITROPHENYLOXY)CARBONYL]PYRIMIDINE: 4-Nitrophenyl chloroformate (1.886 g, 9.38 mmol) was added to a solution of 6-(3,4,5-trifluorophenyl)-1,6-dihydro-2-methoxy-5-acetyl-4-methylpyrimidine (2.80 g, 9.38 mmol) and pyridine (10 mL) in CH₂Cl₂ (200 mL) at 0-5 °C and then the mixture was allowed to warm to room temperature. After 12 h, the solvent was evaporated and the residue was purified by flash chromatography (CH₂Cl₂/EtOAc, 9/1 to

20/3), giving the desired product as a white powder (4.0 g, 92%).

6-(3,4,5-TRIFLUOROPHENYL)-1,2,3,6-TETRAHYDRO-2-OXO-5-ACE
5 TYL-4-METHYL-1-[(4-NITROPHENYLOXY) CARBONYL] PYRIMIDINE:
Aqueous 6 N aqueous HCl (4 mL) was added to a stirring
s o l u t i o n o f
6-(3,4,5-trifluorophenyl)-1,6-dihydro-2-methoxy-
5 - a c e t y l - 4 -
10 methyl-1-[(4-nitrophenyloxy) carbonyl]pyrimidine (4.0 g,
8.63 mmol) in THF (100 mL) at 0-5 °C, and the mixture was
allowed to warm to room temperature. After 2 h, the solvent was evaporated and the product was dried under
vacuum, giving the desired product as a pure single
15 component which was used in the next step without further
purification (3.88 g, 100%).

(+)-1,2,3,6-TETRA HYDRO-1-{N-[4-(4-FLUOROPHENYL)-
20 PIPERIDINE-1-YL]-PROPYL} CARBOXAMIDO-5-ACETYL-2-
OXO-6-(3,4,5-TRI FLUORO PHENYL)-4-METHYL PYRIMIDINE
HYDROCHLORIDE: ^1H NMR δ 7.20-6.86 (m, 6 H), 6.64 (s, 1 H),
5.56 (s, 1 H), 3.70-3.80 (m, 2 H), 3.43-3.35 (m, 2 H),
3.19-2.98 (m, 2 H), 2.40 (s, 3 H), 2.28 (s, 3 H),
2.50-1.60 (m, 8 H).

25

Example 13

N1-[4-([4-(DIBUTYLAMINO)BENZYL]AMINOMETHYL)CYCLOHEXYL]-1
-NAPHTH-AMIDE: ^1H NMR δ 8.26 (dd, 1 H, J=2.1, 7.2 Hz), 7.87
(m, 2 H), 7.51 (m, 2 H), 7.40 (apparent t, 1 H, J=7.8 Hz),
30 7.17 (d, 1 H, J=8.7 Hz), 6.61 (d, 2 H, J=8.7 Hz), 5.94 (d,
1 H, J=8.1 Hz), 4.04 (m, 1 H), 3.76 (m, 1 H), 3.63 (m, 2
H), 3.21 (t, 4 H, J=7.6 Hz average), 2.53 (d, 2 H, J=6.7
Hz), 2.10, ABm, 4 H), 1.55 (p, 4 H, J=7.7 Hz average),
1.34 (sept, 4 H, J=7.6 Hz average), 1.17 (m, 4 H), 0.95

(t, 6 H, J=7.6 Hz average).

Example 14

(+)-1,2,3,6-TETRAHYDRO-1-{N-[4-(1-NAPHTHYL)-PIPERIDIN-1-YL]PROPYL-CARBOXYAMIDO-4-METHOXYMETHYL-6-(3,4-DIFLUOROPHENYL)-2-OXO-PYRIMIDINE-5-CARBOXYLIC ACID METHYL ESTER: mp 168-172 °C; [α]_D = +94.7, (c = 0.25, MeOH); ¹H NMR δ 1.75-1.84 (m, 2H), 1.87-2.01 (m, 4H), 2.14-2.28 (m, 2H), 2.47 (t, J=7.2 Hz, 2H), 3.10 (d, J=11.1 Hz, 2H), 3.28-3.45 (m, 3H), 3.48 (s, 3H), 3.71 (s, 3H), 4.68 (s, 2H), 6.70 (s, 1H), 7.05-7.12 (m, 2H), 7.16-7.24 (m, 1H), 7.42-7.54 (m, 4H), 7.69-7.75 (m, 2H), 7.85 (d, J=11.4 Hz, 1H), 8.09 (d, J=11.1 Hz, 1H), 8.91 (t, J=5.4 Hz, 1H).

15

Example 15

4-(5-FLUORO-2-METHOXY)PHENYL PIPERIDINE: mp 254-258 °C; ¹H NMR δ 1.53-1.68 (m, 2H), 1.79 (d, J=11.7 Hz, 2H), 2.12 (dt, J=2.1 Hz, J=11.7 Hz, 1H), 2.77 (dt, J=1.8 Hz, J=12.3 Hz, 1H), 2.90-3.05 (m, 1H), 3.10-3.22 (m, 2H), 3.68 (s, 1H), 3.79 (s, 3H), 6.72-6.93 (m, 3H). Anal. Calcd. For C₁₂H₁₇NOFCl + 0.14 CH₂Cl₂: C, 56.60; H, 6.76; N, 5.44. Found: C, 56.60; H, 6.92; N, 5.28.

25

(+)-1,2,3,6-TETRAHYDRO-1-{N-[4-(5-FLUORO-2-METHOXY)PHENYLPIPERIDIN-1-YL]PROPYLCARBOXAMIDO-4-METHOXYMETHYL-6-(3,4-DIFLUOROPHENYL)-2-OXOPYRIMIDINE-5-CARBOXYLIC ACID METHYL ESTER: ¹H NMR δ 8.93 (t, 1 H, J=5.4 Hz), 7.76 (br, 1 H), 7.30-6.69 (m, 7 H), 4.69 (s, 2 H), 3.79 (s, 3 H), 3.71 (s, 3 H), 3.48 (s, 3 H), 3.38 (m, 2 H), 3.10-2.80 (m, 3 H), 2.42 (t, 2 H, J=7.2 Hz), 2.07 (dt, 2 H, J=3.0, 8.4 Hz), 2.00-1.60 (m, 6 H).

30

Example 16

(+)-1,2,3,6-TETRAHYDRO-1-{N-[4-HYDROXY-4-(2-PYRIDYL)-PIPERIDIN-1-YL]PROPYL}CARBOXAMIDO-4-METHOXYMETHYL-6-(3,4-DIFLUOROPHENYL)-2-OXOPYRIMIDINE-5-CARBOXYLIC ACID METHYL ESTER: mp 132-135 °C; $[\alpha]_D = +94.7$, ($c = 0.25$, MeOH); ^1H NMR
5 δ 1.47 (d, $J=11.7$ Hz, 2H), 1.74-1.85 (m, 2H), 2.43-2.63 (m, 9H), 2.87 (d, $J=10.2$ Hz, 2H), 3.30-3.47 (m, 2H), 3.49 (s, 3H), 3.71 (s, 3H), 4.69 (s, 2H), 6.69 (s, 1H), 7.04-7.21 (m, 4H), 7.49 (dd, $J=0.6$ Hz, $J=6.9$ Hz, 1H), 7.72 (s, br, 1H), 8.36 (dd, $J=1.2$, 4.8 Hz, 1H), 8.89 (t, $J=5.4$ Hz, 1H).

10

Example 17

1-(3-AMINOPROPYL)-4-[2-PYRIDYL]PYRIDINIUM BROMIDE HYDROBROMIDE: A solution of 2,4'-dipyridyl (25.0 g, 160 mmol) and 3-bromopropyl-amine hydrobromide (35.0 g, 160 mmol) in DMF (60 mL) was heated at 90-95 °C for 10 h. After cooling to room temperature, anhydrous ether (500 mL) was added to the mixture, the resulting white solid was filtered, washed with Et_2O and dried, giving 1-(3-aminopropyl)-4-[2-pyridyl]pyridinium bromide hydrobromide (60 g, 100%). ^1H NMR (DMSO- d_6) δ 2.35-2.44 (m, 2 H), 3.08-3.13 (m, 2 H), 4.76-4.81 (m, 2 H), 7.58 (dd, $J=4.8$ Hz, $J=7.5$ Hz, 1 H), 8.03 (dt, $J=1.8$ Hz, $J=7.8$ Hz, 1 H), 8.32 (d, $J=7.8$ Hz, 1 H), 8.77-8.81 (m, 3 H), 9.12 (d, $J=6.3$ Hz, 2 H). Anal. Calcd. for $\text{C}_{13}\text{H}_{16}\text{N}_3\text{Br} + \text{HBr} + 0.5 \text{H}_2\text{O}$: C, 40.65; H, 4.72; N, 10.94. Found: C, 40.83; H, 4.37; N, 11.05.

3-(3',6'-DIHYDRO-2'-H-[2,4']BIPYRIDINYL-1'-YL)-PROPYLAMINE: NaBH_4 (2 g, 53 mmol) in small portions was added to a solution of 1-(3-aminopropyl)-4-[2-pyridyl]pyridinium bromide hydrobromide (6 g, 16 mmol) in MeOH (150 mL) at 0-5 °C over a period of 2 h. The reaction mixture was stirred overnight at room temperature and then the solvent was evaporated. The residue was suspended in ether (200

mL) and treated with aqueous 50% NaOH solution (100 mL). The ether layer was separated and the aqueous layer was extracted with additional ether (2 X 50 mL). The combined ether extracts were dried over potassium carbonate and the solvent was removed, giving 3-(3',6'-dihydro-2'-H-[2,4']bipyridinyl-1'-yl)-propylamine (3.48 g) as an oil. The crude product was used in the next step immediately without further purification.

10 3-AMINOPROPYL-4-(2-PYRIDYL)PIPERIDINE: A suspension of
 3-(3',6'-dihydro-2'-H-[2,4']bipyridinyl-1'-yl)-propylamine
 (3.48 g crude, 15.9 mmol) and Pearlman's catalyst (1.0 g)
 in MeOH (40 mL) was hydrogenated under 120 psi for 10 h,
 15 after which the reaction mixture was filtered through a
 pad of Celite and the solvent was removed. The residue
 was purified by column chromatography over silica gel (30
 g) [Note: If a large excess of silica gel is used the
 recovery of the product will be very low
 20 (CH₂Cl₂/methanol/2M NH₃ in MeOH, 90/8/4 to 90/40/40). The
 product was obtained as a pale yellow oil (3.21 g, 91%).
¹H NMR δ (CD₃OD) 1.50-1.99 (m, 10 H), 2.02-2.06 (m, 2 H),
 2.37-2.75 (m, 3 H), 3.02-3.06 (br m, 2 H), 7.05-7.09 (m,
 4 H), 7.16 (dt, J=0.9 Hz, J=8.7 Hz, 1 H), 8.48 (dd, J=0.9
 25 Hz, J=4.2 Hz, 1 H).

Part II

5-METHOXCARBONYL-4-METHOXYMETHYL-1,2,3,6-TETRAHYDRO-2-OXO-6-(3,4-DIFLUOROPHENYL)-PYRIMIDINE: Copper(I) oxide (5.06 g, 0.035 mole) and acetic acid (2.05 mL) were added

sequentially to a stirring solution of methyl 4-methoxyacetacetate (50.0 g, 0.351 mol), 3,4-difluorobenzaldehyde (51.4 g, 0.351 mmol), and urea (31.6 g, 0.527 mole) in THF (300 mL) at room temperature, followed by dropwise addition of boron trifluoride diethyl etherate (56.0 mL, 0.456 mole). The mixture was stirred at reflux temperature for 8 h, whereupon TLC (1/1 EtOAc/hexanes) indicated completion of the reaction. The reaction mixture was cooled and poured into a mixture of ice and sodium bicarbonate (100 g) and the resulting mixture was filtered through Celite. The Celite pad was washed with dichloromethane (400 mL). The organic layer was separated from the filtrate and the aqueous layer was extracted with more dichloromethane (3 X 300 mL). The combined organic extracts were dried (sodium sulfate) and the solvent was evaporated. The crude product was purified by flash chromatography (ethyl acetate/hexanes, 1/1; then ethyl acetate), giving the desired product as a pale yellow foam. The foam was triturated with hexanes, giving a white powder (103.3 g, 94%). ^1H NMR δ 3.476 (s, 3H), 3.651 (s, 3H), 4.653 (s, 2H), 5.39 (s, 1H), 6.60 (br s, 1H, NH), 7.00-7.20 (m, 3H), 7.72 (br s, 1H, NH).

(+) -5-METHOXYCARBONYL-4-METHOXYMETHYL-1,2,3,6-TETRAHYDRO-2-OXO-6-(3,4-DIFLUOROPHENYL)-PYRIMIDINE: The racemic intermediate 5-methoxycarbonyl-4-methoxymethyl-1,2,3,6-tetrahydro-2-oxo-6-(3,4-difluorophenyl)pyrimidine was resolved by chiral HPLC [Chiralcel OD 20 X 250 mm #369-703-30604; lambda 254 nm; hexanes/ethanol 90/10 ; 85 mg per injection; retention time of the desired enantiomer: 16.94 min., the first enantiomer peak to elute], giving (+)-5-methoxycarbonyl-4-methoxymethyl-1,2,3,6-tetrahydro-2-oxo-6-(3,4-difluorophenyl)-pyrimidine (40-42 wt% isolation of the desired enantiomer from the

racemate); $[\alpha]_D = +83.8$ ($c = 0.5$, chloroform).

(+) -5-METHOXCARBONYL-4-METHOXYMETHYL-1,2,3,6-TETRAHYDRO-2-OXO-6-(3,4-DIFLUOROPHENYL)-1-[(4-NITROPHENYLOXY) CARBO NYL] PYRIMIDINE: A solution of lithium hexamethyldisilazide in THF (1M, 18.0 mL, 18.0 mmol) was added over 2-3 min. to a solution of (+)-5-methoxycarbonyl-4-methoxymethyl-1,2,3,6-tetrahydro-2-oxo-6-(3,4-difluorophenyl)-pyrimidine (1.98 g, 6.34 mmol) in anhydrous THF (20 mL) at -78 °C under argon atmosphere and the mixture was stirred for 10 min. The resulting solution was added over 6 min., via a cannula, to a stirred solution of 4-nitrophenyl chloroformate (4.47 g, 22.2 mmol) in THF (20 mL) at -78 °C. The mixture was stirred for an additional 10 min. and the mixture was poured onto ice (50 g) and extracted with chloroform (2 X 50 mL). The combined extracts were dried (sodium sulfate) and the solvent evaporated. The residue was purified by flash chromatography (hexanes/ethyl acetate, 4/1 to 3.5/1), giving the product as a yellow syrup, which on trituration with hexanes became a white powder (2.40 g, 79%). ^1H NMR δ 3.52 (s, 3H), 3.74 (s, 3H), 4.65-4.80 (q, $J=16.5$ Hz, 2H), 6.32 (s, 1H), 7.10-7.30 (m, 4H), 7.36 (d, $J=9$ Hz, 2H), 8.27 (d, $J=9$ Hz, 2H).

(+) -6-(3,4-DIFLUOROPHENYL)-1-{N-[4-(2-PYRIDYL) PIPERIDIN-1-YL] CARBOXYAMIDO-5-METHOXCARBONYL-4-METHOXYMETHYL-2-OXO-1,2,3,6-TETRAHYDROPRIMIDINE DIHYDROCHLORIDE: A solution of (+)-5-methoxycarbonyl-4-methoxymethyl-1,2,3,6-tetrahydro-2-oxo-6-(3,4-difluorophenyl)-1-[(4-nitrophenyloxy) carbonyl]pyrimidine (2.38 g, 5 mmol), 3-aminopropyl-4-(2-pyridyl)piperidine (1.21 g, 5.5 mmol) in THF (20 mL) was stirred at room temperature for 12 h.

The solvent was evaporated and the residue was re-dissolved in ethyl acetate (100 mL). The resulting solution was washed with ice-cold 1 N NaOH (4 X 50 mL), brine (2 X 50 mL) and dried over potassium carbonate. The solvent was evaporated *in vacuo* and the residue was purified by flash chromatography (dichloromethane/MeOH/2 M ammonia in MeOH, 980/10/10 to 940/30/30), giving a clean fraction of the desired product (2.45 g, 88%) as a foam and a slightly impure fraction (0.30 g, 10%). ¹H NMR δ 1.60-2.00 (m, 6H), 2.05-2.15 (m, 2H), 2.38-2.43 (br t, 2H), 2.65-2.80 (m, 1H), 3.05-3.06 (br d, 2H), 3.30-3.45 (m, 2H), 3.48 (s, 3H), 3.704 (s, 3H), 4.68 (s, 2H), 6.68 (s, 1H), 7.05-7.20 (m, 5H), 7.58-7.63 (dt, 1H), 7.70 (s, 1H, NH), 8.50-8.52 (dd, 1H), 8.88 (br t, 1H).

The HCl salt was prepared by treatment of a solution of the free base in ether with 1 N HCl in ether. The white powder was dried under reduced pressure: ¹H NMR δ 2.05-2.20 (m, 4H), 2.77-2.88 (m, 2H), 3.00-3.20 (m, 4H), 3.35-3.47 (m, 2H), 3.47 (s, 3H), 3.64-3.70 (m, 2H), 3.71 (s, 3H), 4.05 (br t, 1H), 4.67 (s, 2H), 6.59 (s, 1H), 7.05-7.20 (m, 3H), 7.79 (t, 1H), 8.00 (d, 1H), 8.43 (dt, 1H), 8.96 (br t, 1H, NH), 12.4 (br s, 1H). m.p. 188-191 °C; [α]_D = +141.13 (c = 0.265, MeOH); Anal. Calcd. for C₂₈H₃₄N₅O₅F₂Cl + 0.6 H₂O:C, 52.36; H, 5.84; N, 10.90. Found: C, 52.24; H, 5.96; N, 10.80. (Note: NMR analysis of this product did not show the presence of any water. However, it was noted by the lab that performed the elemental analysis that this sample gains weight during handling by absorbing water from the atmosphere).

30

Example 18

(1)-1,2,3,6-TETRAHYDRO-1-{N-[4-(ISO BENZOFURAN) PIPERIDINE-1-YL]-PROPYL}CARBOXAMIDO-5-METHOXYCARBONYL-2-OXO-6-(3,4-

BENZOFURAZAN) - 4-METHYL PYRIMIDINE HYDROCHLORIDE

4-(3,4-BENZOFURAZAN)-6-METHYL-2-OXO-3-[3-(4-SPIRO[ISOBE
N Z O -
5 FURAN-1(3H),4'-PIPERIDINE]PROPYL]-1,2,3,4-TETRAHYDROPYRI
MIDINE-5-CARBOXYLIC ACID METHYL ESTER :
1-(3-Aminopropyl)-4-spiro[iso-benzofuran-1
(3H),4'-piperidine] (0.028 g, 0.110 mmol) was added to
(±)-6-(benzofurazan)-1,6-dihydro-
10 2-methoxy-5-methoxycarbonyl-4-methyl-1-(4-nitrophenoxy)
carbonylpyrimidine (0.047 g, 0.100 mmol) in dry
dichloromethane (10 mL) and the solution was stirred at
room temperature for 24 h. Aquesous 6 N HCl (2 mL) was
added to the reaction mixture which was stirred for
15 another 1 h. The reaction mixture was basified with
aqueous 10% KOH solution (pH = 9) and extracted into
dichloromethane (3 x 10 mL). The organic layer was dried
over sodium sulfate, filtered and concentrated. The crude
product was purified by flash chromatography (EtOAc/ MeOH,
20 4.5/0.5), giving the desired product (41.0 mg, 73 %) as
a syrup: ^1H NMR δ 1.76-1.81 (m, 7 H), 1.94-2.04 (m, 6 H),
2.32-2.48 (m, 1 H), 2.83 (d, $J=10.6$ Hz, 2 H), 3.36-3.43
(m, 2 H), 3.75 (s, 3 H), 5.05 (s, 2 H), 6.83 (s, 1 H),
7.07-7.27 (m, 4 H), 7.54 (d, $J=9.5$ Hz, 1 H), 7.69 (s, 1
25 H), 7.78 (d, $J=9.5$ Hz, 1 H), 8.85 (d, $J=5.2$ Hz, 1 H).

HCl in ether (1 N, 5 mL) was added to the free base (0.041
g, 0.073 mmol) in dichloromethane (4 mL), and the solution
was concentrated under reduced pressure. The product was
30 recrystallized from ether, giving the hydrochloride salt
as a pale yellow solid (42.0 mg, 96 %); mp 180-182 °C;
Anal. Calcd. for $\text{C}_{29}\text{H}_{34}\text{N}_6\text{O}_6\text{Cl} + 0.5$ moles H_2O : C, 57.47; H,
5.65; N, 13.87. Found: C, 57.42; H, 5.71; N, 13.70.

2 - (3 , 4 - DIFLUOROPHENYL) 4 , 5 - DIHYDROIMIDAZOLE - 1 - CARBOXYLIC

A

C

I

D

{ 3 - [4 - PHENYL - 4 - (4 - BROMO - 5 - METHYLTHIOPHENEN - 2 - YL)] - PROPYL } -

AMIDE: Anal. Calcd. for C₃₀H₃₀N₄O₅ClF₃ + HCl + 1.5 H₂O: C,

5 55.26; H, 6.03; N, 8.59. Found: C, 55.29; H, 5.95; N,
8.39.

Example 20

4 - (3 , 4 - DIFLUOROPHENYL) - 6 - METHYL - 2 - OXO - 3 - { [3 - (4 - SPIRO [ISOB

10 ENZO - FURAN - 1 (3H) , 4 ' - PIPERIDINE] PROPYL } - 1 , 2 , 3 , 4 -

TETRAHYDROPRIMIDINE - 5 - CARBOXYLIC ACID METHYL ESTER

For the preparation of the ether piperidine precursor of
the compound of Example 20, refer to W.E.Parham *et al*, *J.
Org. Chem.* (1976) 41, 2268.

15

1 - TERT - BUTOXYSYCARBONYL - 3 - (4 - SPIRO [ISO BENZOFURAN - 1 (3H) , 4 ' -

PIPERIDINE]) PROPYLAMINE: N - (*tert* - butoxycarbonyl) - 3 - bromo -
propylamine (0.772 g, 3.27 mmol) and potassium carbonate

20 (0.904 g, 6.54 mmol) were added to a stirring solution of
the amine (0.566 g, 3.27 mmol) in dioxane (20 mL) and the
reaction mixture was heated at reflux temperature for 24

h. The reaction mixture was cooled to room temperature,
concentrated and partitioned between chloroform (40 mL)
and water (5 mL). The organic layer was dried over sodium

25 sulfate, filtered and concentrated. The crude product was
purified by column chromatography (ethyl acetate /
methanol, 4.5/0.5), giving the desired product (0.856 g,
79 %) as a colorless oil; ¹H NMR δ 1.45 (s, 9 H), 1.63 - 2.04

(m, 6 H), 2.33 - 2.52 (m, 4 H), 2.87 (d, J = 11.0 Hz, 2 H),

30 3.2 (br s, 2 H), 5.07 (s, 2 H), 5.6 (br s, 1 H), 7.13 - 7.28
(m, 4 H).

3 - (4 - S P I R O [I S O B E N Z O -
FURAN - 1 (3H) , 4 ' - PIPERIDINE]) PROPYLAMINE: Trifluoroacetic

acid (1 mL) was added to 1-tert-butoxycarbonyl
5 3-(4-spiro[isobenzo-furan-1(3H), 4'-
piperidine])propylamine (0.500 g, 1.51 mmol) in
dichloromethane (5 mL) and the solution was stirred at
room temperature for 1 h. The reaction mixture was
concentrated, neutralized with 10 % KOH solution and
extracted into dichloromethane (25 mL). The organic layer
was dried over sodium sulfate, filtered and concentrated,
giving the desired amine (0.340 g, 98%) which was used in
10 the subsequent step without further purification.

4-(3,4-DIFLUORPHENYL)-6-METHYL-2-OXO-3-[3-(4-SPIRO[ISOB
ENZO-FURAN-1(3H), 4'-PIPERIDINE]PROPYL]-1,2,3,4-
TETRAHYDROPRIMIDINE-5-CARBOXYLIC ACID METHYL ESTER:
15 3-(4-spiro[isobenzo-furan-1(3H), 4'-piperidine])propylamine
(0.0319 g, 0.123 mmol) was added to
(±)-6-(3,4-Difluorophenyl)-1,6-dihydro-
2-methoxy-5-methoxycarbonyl-4-methyl-1-(4-nitrophenoxy)c
arbonylpyrimidine (0.052 g, 0.112 mmol) in dry
20 dichloromethane (10 mL) and the solution was stirred at
room temperature for 24 h. Aqueous 6 N HCl (2 mL) was
added and the reaction mixture was stirred for an
additional 1 h. After neutralization with 10% aqueous KOH
solution, the reaction mixture was extracted with
25 dichloromethane (3 x 10 mL). The organic layer was dried
over sodium sulfate, filtered and concentrated. The crude
product was purified by flash chromatography (EtOAc/ MeOH,
4.5/0.5), giving the desired product (0.040 g, 64 %) as a
syrup; 1H-NMR δ 1.73-1.78 (m, 7 H), 1.93-2.04 (m, 2 H),
30 2.33-2.48 (m, 6 H), 2.83 (d, J=11.8 Hz, 2 H), 3.35-3.41
(m, 2 H), 3.71 (s, 3 H), 5.06 (s, 2 H), 6.75 (s, 1 H),
7.04-7.26 (m, 7 H), 8.82 (t, J=5.1 Hz, 1 H).

35 A solution of 1 N HCl in ether (5 mL) was added to the
free base (0.040 g, 0.072 mmol) in dichloromethane (4 mL)

and the solution was concentrated *in vacuo*. The product was recrystallized from ether, giving the dihydrochloride as a pale yellow solid (0.042 g, 99 %); mp 178-182 °C; Anal. Calcd. for $C_{29}H_{34}F_2N_4O_5Cl_2 + 0.6 H_2O$: C, 57.87; H, 5.73, N 9.31. Found: C, 58.11; H 5.90; N 8.95.

5 **Example 21**

10 1,2,3,6-TETRAHYDRO-1-{N-[4-(DIHYDROINDENE)-1-YL]PROPYL}CARBOXYAMIDO-5-METHOXYSARONYL-2-OXO-6-(3,4-BENZOFURAZAN)-4-METHYLPYRIMIDINE

15 For the preparation of the indane piperidine precursor of the compound of Example 21, refer to M.S.Chambers *J. Med. Chem.* (1992) 35,2033.

20 N-(tert-butoxycarbonyl)3-(4-spiro[isobenzo-furan-1(3H),4'-piperidine])propylamine (1.10 g, 4.64 mmol) and potassium carbonate (1.17 g, 8.44 mmol) were added to a stirring solution of the amine (0.790 g, 4.22 mmol) in dioxane (20 mL), and the resulting solution was heated at reflux temperature for 24 h. The reaction mixture was cooled to room temperature, concentrated and partitioned between chloroform (40 mL) and water (5 mL). The organic layer was dried over sodium sulfate, filtered and concentrated. 25 The crude product was purified by column chromatography (ethyl acetate/ methanol, 4.5/0.5), giving the desired product (0.886 g, 61 %) as a colorless oil; 1H NMR δ 1.46 (s, 9 H), 1.55 (d, J = 11.3 Hz, 2 H), 1.69 (t, J = 6.3 Hz, 2 H), 1.88-2.47 (m, 6 H), 2.47 (t, J = 6.3 Hz, 2 H), 2.88 (t, J = 3.3 Hz, 4 H), 3.23 (d, J = 5.6 Hz, 2 H), 5.85 (br s, 1 H), 7.18 (s, 4 H).

30 Trifluoroacetic acid (1 mL) was added to 1-tert-butoxycarbonyl-3-(4-spiro[isobenzo-

furan-1(3H),4'-piperidine])propylamine(0.180 g, 0.52 mmol) in dichloromethane (5 ml) and the resulting solution was stirred at room temperature for 1 hour. The solution was concentrated, neutralized with 10% KOH solution and extracted into dichloromethane (25 ml). The organic layer was dried over sodium sulfate, filtered and concentrated, giving propylamine (0.156 g, 100%) which was used in the subsequent step without further purification.

(\pm) -4- (3,4-BENZOFURAZAN)-6-METHYL-2-OXO-3-{SPIRO[1H-INDANE-1,4'-PIPERIDINE]PROPYL}-1,2,3,4-TETRAHYDROPYRIMIDINE-5-CARBOXYLIC ACID METHYL ESTER HYDROCHLORIDE: To (\pm) -4- (3,4-benzofurazan)-1,6- dihydro-2-methoxy-5-methoxycarbonyl-4-methyl-1-(4-nitrophenoxy)-carbonylpyrimidine (0.059 g, 0.126 mmol) in dry dichloromethane (10 mL), 1-(3-aminopropyl)spiro[1H-indane-1,4'- piperidine] (0.062 g, 0.252 mmol) was added and the solution was stirred at room temperature for 24 h. The reaction mixture was stirred for another 1 h after addition of 2 mL of 6N HCl. The reaction mixture was basified with 10% aqueous KOH solution (pH = 9) and extracted with dichloromethane (3 x 10 mL). The combined organic extracts were dried over sodium sulfate, filtered and concentrated. The crude product was purified by flash chromatography (EtOAc/ MeOH, 4.5/0.5), giving 0.070 g (100%) of the desired product as a syrup: ^1H NMR δ 1.51 (d, $J=12.5$ Hz, 2 H), 1.76-2.08 (m, 4 H), 2.12 (t, $J=10.3$ Hz, 2 H), 2.45 (s, 5 H), 2.86-2.91 (m, 4 H), 3.30-3.45 (m, 2 H), 3.75 (s, 3 H), 6.83 (s, 1 H), 7.02 (br s, 1 H), 7.0 (m, 4 H), 7.54 (d, $J=9.6$ Hz, 1 H), 7.69 (s, 1 H), 7.78 (d, $J=9.2$ Hz, 1 H), 8.84, (t, $J=5.2$ Hz, 1 H).

To the free base (0.070 g, 0.125 mmol) in 4 mL of dichloromethane, 5 mL of 1 N HCl in ether was added, and

the solution was concentrated under reduced pressure. Recrystallization from ether gave 0.088 g (100 %) of (\pm) -4- (3,4-benzofurazan)-6-methyl-2-oxo-3-{spiro[1H-indan-1,4'-piperidine]propyl}-1,2,3,4-tetrahydropyrimidine-5-carboxylic acid methyl ester hydrochloride as a white solid: m.p. 155-157 °C; Anal. Calcd. for C₃₀H₃₈N₆O₅Cl: C, 57.12; H, 5.76; N, 13.33. Found: C, 57.40; H, 5.96; N, 13.02.

10

Example 22

(+)-1,2,3,6-TETRAHYDRO-1-{N-[4-(BENZO-4',5' (H) FURAN) PIPERIDIN-1-YL] PROPYL} CARBOXAMIDO-4-ETHYL-6-(3,4-DIFLUOROPHENYL)-2-OXO-PYRIMIDINE-5-CARBOXAMIDE HYDROCHLORIDE: DMAP · ECD (0.250 mmol, 0.050 g) was added to a stirred mixture of (+)-1,2,3,6-tetrahydro-1-{N-[4-(benzo-4',5' (h) furan)-piperidin-1-yl]propyl}carbox-amido-4-ethyl-6-(3,4-difluorophenyl)-2-oxo-pyrimidine-5-carboxyl-ic acid hydrochloride (0.100 mmol, 0.055 g) and N-methylmorpholine (0.330 mL) in dry dichloromethane (10 mL). The resulting mixture was stirred at room temperature for 1 h and quenched with NH₃. The reaction mixture was stirred at room temperature overnight, concentrated and chromatographed, giving the desired product. The HCl salt was prepared by the addition of HCl in ether to a solution of the product in dichloromethane, followed by evaporation of the solvents. Anal. Calc. For C₂₉H₃₃N₅O₄F₂ + HCl + 0.7 CHCl₃ : C, 52.96; H, 5.29; N, 9.40. Found: C, 52.81; H, 5.69; N, 8.97.

20

25

30

Example 23

(1)-1,2,3,6-TETRAHYDRO-1-{N-[4-(3,4-DIHYDRO-2-OXOSPIRONAPHTHALENE-1(2H))-PIPERIDINE-1-YL] PROPYL}CARBOXAMIDO-5-METHOXCARBONYL-2-OXO-6-(3,4-BENZOFURAZAN)-4-

35

METHYLPYRIMIDINE HYDROCHLORIDE

1 - (3-TERT-BUTOXYCARBONYLAMINOPROPYL) SPIRO [ISOCHROMAN-3,4
'PIPERIDIN] -1-ONE: To a stirred solution of
5 spiro [piperidine-4,1'-tetralin] To a stirred solution of
spiro [isochroman-3,4'-piperidin]-1-one (K.Hashigaki et al.
Chem.Pharm.Bull. (1984) 32, 3568.) (0.587 g, 2.58 mmol)
in dioxane (20 mL), N-(tert-
butoxycarbonyl)-3-bromopropylamine (0.615 g, 2.84 mmol)
10 and potassium carbonate (0.714 g, 5.17 mmol) were added
and the solution was refluxed for 24 h. The reaction
mixture was cooled to room temperature, concentrated and
partitioned between 40 mL chloroform and 5 mL water. The
organic layer was dried over sodium sulfate, filtered and
concentrated. The crude product was purified by column
15 chromatography (ethyl acetate/ methanol, 4.5/0.5) to yield
0.465 g (47 %) of the desired product as a colorless oil;
¹H NMR δ 1.45 (s, 9 H), 1.64-2.18 (m, 7 H), 2.45-2.84 (m, 6
H), 3.19-3.95 (m, 4 H), 6.01 (br s, 1 H), 7.13-7.26 (m, 3
H), 7.42 (d, J=7.7 H).

Step B. 1 - (3-AMINOPROPYL) SPIRO [ISOCHROMAN-3,4'PIPERIDIN] -
1-ONE: To 1-(3-tert-Butoxycarbonylaminopropyl)-
25 spiro [isochroman-3,4'-piperidin]-1-one (0.144 g, 0.375
mmol) in 5 mL of dichloromethane, 1 mL of trifluoroacetic
acid was added and the solution stirred at room
temperature for 1 h. The solution was concentrated,
neutralized with 10 % KOH solution and extracted into 25
mL of dichloromethane. The organic layer was dried over
30 sodium sulfate, filtered and concentrated, giving 0.110 g
(100%) of the product which was used as such for the
subsequent step.

(±)-4-(3,4-BENZOFURAZAN)-6-METHYL-2-OXO-3-{(SPIRO[ISOCHR
35 OMAN- 3,4'-PIPERIDIN]-1-ONE) PROPYL}-1,2,3,4-

TETRAHYDROPYRIMIDINE-5-CARBOXYL-IC ACID METHYL ESTER: To
5 (\pm) -4-(3,4-Benzofurazan)-1,6-dihydro-2-methoxy-5-methoxy carbonyl-4-methyl-1-(4-nitrophenoxy)-carbonylpyrimidine (40.0 mg, 0.0865 mmol) in 10 mL of dry dichloromethane,
10 spiro[isochroman-3,4'piperidin]-1-one (44.0 mg, 0.173 mmol) was added and the solution was stirred at room temperature for 24 h. The reaction mixture was stirred for another 1 h after addition of 2 mL of 6N HCl. The reaction mixture was basified with 10% aqueous KOH
15 solution (pH = 9) and extracted into dichloromethane (3 x 10 mL). The organic layer was dried over sodium sulfate, filtered and concentrated. The crude product was purified by flash chromatography (EtOAc/ MeOH, 4.5/0.5), giving 50.0 mg (100%) of the desired product as a syrup: ^1H NMR
20 δ 1.67-2.13 (m, 8 H), 2.45 (m, 5 H), 2.70 (t, $J=7.4$ Hz, 2 H), 2.72-2.75 (m, 2 H), 3.19 (t, $J=7.4$ Hz, 2 H), 3.34-3.45 (m, 2 H), 3.75 (s, 3 H), 6.82 (s, 1 H), 6.87 (s, 1 H), 7.13-7.44 (m, 3 H), 7.54 (d, $J=9.6$ Hz, 1 H), 7.43 (d, $J=7.4$ Hz, 1 H), 7.69 (s, 1 H), 7.79 (d, $J=9.6$ Hz, 1 H),
25 8.87 (t, $J=5.2$ Hz, 1 H).

To the free base (50.0 mg, 0.084 mmol) in 4 mL of dichloromethane, 5 mL of 1 N HCl in ether was added, and the solution concentrated under reduced pressure.
30 Recrystallization from ether gave 30.0 mg (86 %) of the product as a white solid: m.p. 165-167 °C; Anal. Calcd. for $\text{C}_{31}\text{H}_{36}\text{N}_6\text{O}_6\text{Cl} + 1.5 \text{ H}_2\text{O}$: C, 57.81; H, 5.95. Found: C, 57.75; H, 5.91.

35

Example 24

(1) -1,2,3,6-TETRAHYDRO-1-{N-[4-(3,4-DIHYDRO-2-OXOSPIRONAPHTHALENE-1(2H))-PIPERIDINE-1-YL]PROPYL}CARBOXAMIDO-5-METHOXY-CARBONYL-2-OXO-6-(3,4-DIFLUOROPHENYL)-4-METHYLPYRIMIDINE

(\pm) -4- (3,4-DIFLUOROPHENYL) -6-METHYL-2-OXO-3-{(SPIRO[ISOC
HROMAN- 3,4'PIPERIDIN]-1-ONE) PROPYL}-1,2,3,4-
TETRAHYDROPYRIMIDINE-5- CARBOXYLIC ACID METHYL ESTER: To
(\pm) -4- (3,4-Difluorophenyl)- 1,6-dihydro-2-methoxy-5-
methoxycarbonyl-4-methyl-1-(4-nitrophen-
oxy)carbonylpyrimidine (40.0 mg, 0.0865 mmol) in 10 mL of
dry dichloromethane, spiro[isochroman-3,4'piperidin]-1-one
(44.0 mg, 0.173 mmol) was added and the solution was
stirred at room temperature for 24 h. The reaction
mixture was stirred for another 1 h after addition of 2 mL
of 6N HCl. The reaction mixture was basified with 10%
aqueous KOH solution (pH = 9) and extracted into
dichloromethane (3 x 10 mL). The organic layer was dried
over sodium sulfate, filtered and concentrated. The crude
product was purified by flash chromatography (EtOAc/ MeOH,
4.5 / 0.5), giving 45.0 mg (90%) of
(\pm) -4- (3,4-difluorophenyl)- 6-methyl-2-oxo-3-
{(spiro[isochroman-3,4'piperidin]-1-one)propyl}-1,2,3,4-
tetrahydropyrimidine-5-carboxylic acid methyl ester as a
syrup; ^1H NMR δ 1.75-1.94 (m, 9H), 2.05-2.13 (m, 4 H),
2.36-2.41 (m, 5 H), 2.70 (t, $J=7.35$ Hz, 2 H), 2.77 (m, 2
H), 3.19 (t, $J=7.4$ Hz, 2 H), 3.39-3.43 (m, 2 H), 6.69 (s,
1 H), 7.04-7.45 (m, 8 H), 8.82 (t, $J=5.2$ Hz, 1 H).

To the free base (45.0 g, 0.077 mmol) in 4 mL of
dichloromethane, 5 mL of 1 N HCl in ether was added, and
the solution was concentrated *in vacuo*. Recrystallization
from ether gave 0.050 g (100%) of
(\pm) -4- (3,4-difluorophenyl)-6-
methyl-2-oxo-3-{(spiro-[isochroman-3,4'piperidin]-
1-one)propyl}-1,2,3,4-tetrahydro-pyrimidine-5-carboxylic
acid methyl ester hydrochloride as a white solid: m.p.
150-152 °C; Anal. Calcd. for $\text{C}_{31}\text{H}_{38}\text{F}_2\text{N}_4\text{OCl} + 2 \text{H}_2\text{O}$: C, 56.49;
H, 5.96. Found: C, 56.40; H, 5.95.

Example 25

5-[(Z)-1-(1-ETHYL-2,2,4-TRIMETHYL-1,2-DIHYDRO-6-QUINOLINYL)-METHYLIDENE]-2-THIOXO-1,3-TIAZOLAN-4-ONE

5 Example 26

1- [BIS (4-FLUOROPHENYL) METHYL] -4- (3-PHENYL-2-PROPYENYL) PIPERAZINE

Example 27

10 4- [(4 -IMIDAZO [1 , 2 -A] PYRIDIN-2-YLPHENYL) IMINO] METHYL-5-METHYL-1,3-BENZENEDIOL

Example 28

1-[3-(4-CHLOROBENZOYL)]PROPYL-4-BENZAMIDOPIPERIDINE

15 P r e p a r a t i o n o f
1-[3-(4-chlorobenzoyl)propyl]-4-benzamidopiperidine

1-[3-(4-CHLOROBENZOYL) PROPYL]-4-BENZAMIDOPIPERIDINE: A
 mixture of 3-(4-chlorobenzol)propyl bromide (640 mg, 2.45 mmol), 4-benzamidopiperidine (500 mg, 2.45 mmol) and K_2CO_3 (1.01 g, 7.34 mmol) in 50 ml of acetone was heated at reflux temperature for 48 h. The cooled reaction mixture was filtered to remove the solids, concentrated *in vacuo*, giving a yellow solid, which was purified by chromatography (MeOH/CHCl₃, 5/95). The product (320 mg, 33.9%) was isolated as a white powder: ¹H NMR δ 1.46 (dq, J1=1.0 Hz, J2=8.4 Hz, 2H), 1.90-2.10 (m, 4H), 2.16 (m, 2H), 2.43 (t, J=6.9 Hz, 2H), 2.80-2.90 (m, 2H), 2.97 (t, J=6.9 Hz, 2H), 3.97 (m, 1H), 5.92 (d, J=7.8 Hz, 1H, N-H), 7.40-8.00 (m, 9H). The product was converted to the HCl salt and recrystallized from MeOH/Et₂O, m.p. 243-244 °C; Anal. Calcd for C₂₂H₂₅ClN₂O₂ + HCl + H₂O: C, 60.15; H, 6.37; N, 6.37; Found: C, 60.18; H, 6.34; N, 6.29.

Example 29

4 - [4 - (4 - CHLOROPHENYL) - 4 - HYDROXY - 1 - PIPERIDINYL] - 1 - (4 - CHLOROPHENYL) - 1 - BUTANONE

5 **Example 30**

N-METHYL-8 - [4 - (4 - FLUOROPHENYL) - 4 - OXOBUTYL] - 1 - PHENYL-1,3,8-TRI-AZASPIRO-[4.5]DECAN-4-ONE

10 **Example 31**

1H-1,2,3-BENZOTRIAZOL-1-YL (2-NITROPHENYL) SULFONE

15 **Example 32**

(1) -1,2,3,6-TETRAHYDRO-1-{N-[4-(DIHYDROINDENE)-1-YL]PROPYLYL} L }

15 CARBOXAMIDO-5-METHOXYSPIRO[1H-INDANE-1,4'-PIPERIDINE]

1 - (3-TERT-BUTOXYCARBONYLAMINOPROPYL) SPIRO[1H-INDANE-1,4'-PIPERIDINE]: To a stirred solution of spiro[1H-indane-1,4'-piperidine] (M.S.Chambers *et al.* *J. Med. Chem.* 1992) 35, 2033.) (0.790 g, 4.22 mmol) in dioxane (20 mL),

20 N-(tert-butoxy-carbonyl)-3-bromopropylamine (1.1 g, 4.64 mmol) and potassium carbonate (1.17 g, 8.44 mmol) were added and the resulting solution was heated at reflux temperature for 24 h. The reaction mixture was cooled to room temperature, concentrated and partitioned between 40 mL of chloroform and 5 mL of water. The organic layer was dried over sodium sulfate, filtered and concentrated. The crude product was purified by column chromatography (ethyl acetate/ methanol, 4.5/0.5) to yield 0.886 g (61 %) of the required product as a colorless oil:

30 ^1H NMR δ 1.46 (s, 9 H), 1.55 (d, $J=11.3$ Hz, 2 H), 1.69 (t, $J=6.3$ Hz, 2 H), 1.88-2.47 (m, 6 H), 2.47 (t, $J=6.3$ Hz, 2 H), 2.88 (t, $J=3.3$ Hz, 4 H), 3.23 (d, $J=5.6$ Hz, 2 H), 5.85 (br s, 1 H), 7.18 (s, 4 H).

1 - (3 - AMINOPROPYL) SPIRO [1H-INDANE-1, 4' - PIPERIDINE] : To
1 - (3 - tert - Butoxycarbonylaminopropyl) spiro [1H-indane-1, 4' -
piperidine] (0.180 g, 0.52 mmol) in 5 mL of dichloromethane, 1 mL of trifluoroacetic acid was added
5 and the solution stirred at room temperature for 1 h. The solution was concentrated, neutralized with 10 % KOH
solution and extracted into 25 mL of dichloromethane. The organic layer was dried over sodium sulfate, filtered and
concentrated, giving 0.156 g (100%) of the product which
10 was used as such for the subsequent step.

(\pm) -4 - (3, 4 - DIFLUORO) -6 - METHYL -2 - OXO -3 - {SPIRO [1H-INDANE-1,
, 4' - PIPERIDINE] PROPYL} -1, 2, 3, 4 - TETRAHYDROPRIMIDINE -5 - CARBOXYLIC ACID METHYL ESTER : To
15 (\pm) -4 - (3, 4 - difluoro) 1, 6 - dihydro -
2 - methoxy - 5 - methoxycarbonyl - 4 - methyl -1 -
(4 - nitrophenoxy) carbonylpyrimidine (50.0 g, 0.108 mmol) in
10 mL of dry dichloromethane, 1 - (3 - aminopropyl) spiro [1H-indane-1, 4' - piperidine] (53.0 mg,
20 0.216 mmol) was added and the solution was stirred at room temperature for 24 h. The reaction mixture was stirred
for another 1 h after addition of 2 mL of 6N HCl. The reaction mixture was basified with 10% aqueous KOH
solution (pH = 9) and extracted into dichloromethane (3 x
25 10 mL). The organic layer was dried over sodium sulfate, filtered and concentrated. The crude product was purified
by flash chromatography (EtOAc/ MeOH, 4.5/0.5), giving
60.0 mg (100%) of the product as a syrup: ^1H NMR δ 1.52 (d,
J=13.2 Hz, 2 H), 1.70-2.07 (m, 8 H), 2.12 (t, J=10.3 Hz,
30 2 H), 2.42 (s, 4 H), 2.86-2.91 (m, 3 H), 3.32-3.43 (m, 2 H), 3.72 (s, 3 H), 6.71 (s, 1 H), 6.81 (br s, 1 H),
7.04-7.19 (m, 7 H), 8.82 (t, J=5.2 Hz, 1 H).

To the free base (0.060 g, 0.108 mmol) in 4 mL of dichloromethane, 5 mL of 1 N HCl in ether was added, and
35

the solution was concentrated under reduced pressure. Recrystallization from ether gave 0.070 g (100%) of the product as a white solid; m.p. 150-153 °C; Anal. Calcd. for C₃₀H₃₆F₂N₄O₆Cl: C, 54.86; H, 5.53; N, 8.54. Found: C, 54.96; H, 5.57; N, 8.27.

5 **Example 33**

10 (+)-1,2,3,6-TETRAHYDRO-1-{N-[4-(3,4,5-TRIFLUORO)-PHENYL-
PIPERIDIN-1-YL]PROPYL}CARBOXAMIDO-4-METHOXYPYRIMIDINE-2-OXOCARBOXYLIC ACID METHYL
15 ESTER: mp 0°C; [α]_D = +123.0, (c = 0.15, MeOH); ¹H NMR δ 1.70-1.82 (m, 6H), 1.97-2.08 (m, 2H), 2.40 (t, J=6.9 Hz, 2H), 2.74-2.87 (m, 1H), 3.01 (d, J=11.1 Hz, 2H), 3.29-3.40 (m, 2H), 3.49 (s, 3H), 3.71 (s, 3H), 4.69 (s, 2H), 6.68 (s, 1H), 6.88-6.95 (m, 2H), 7.05-7.11 (m, 2H), 7.15-7.22 (m, 1H), 7.71 (s, 1H), 8.90 (t, J=5.4 Hz, 1H).

20 **Example 34**

25 (+)-1,2,3,6-TETRAHYDRO-1-{N-[2-(S)-METHYL]-4-(2-NITROPHENYL)PIPERAZIN-1-YL]PROPYL}-CARBOXAMIDO-4-METHYL-6-(3,4-DIFLUOROPHENYL)-2-OXO-PYRIMIDINE

30 (S)-(+)-3-METHYL-1-(2-NITROPHENYL)-PIPERAZINE: To a solution of 2-bromonitrobenzene (0.600 g, 3.00 mmol) in 1,4-dioxane (15 mL) was added (S)-(+)-2-methylpiperazine (0.500 g, 0.500 mmol) and powdered K₂CO₃ (15.0 mmol, 1.50 g) and the resulting suspension was heated at reflux for 10 h. After the suspension was cooled, it was filtered through a sintered glass funnel and the solvent was removed *in vacuo*. The resulting residue was purified by column chromatography (1/1 hexane/EtOAc followed by 4/1

E t O A c / M e O H) , giving
(S)-(+)-3-methyl-1-(2-nitrophenyl)-piperazine as an orange
oil (0.53 g, 80%).

5 (+)-1,2,3,6-TETRAHYDRO-1-{N-[2-(S)-METHYL]-4-(2-NITROPHE
NYL)PIPERAZIN-1YL]PROPYL}-CARBOXAMIDO-4-METHYL-6-(3,4-DI
FLUOROPHENYL)-2-OXO-PYRIMIDINE: To a solution of (+)-1-
(3-bromo-propyl carbamoyl)-
10 6-(3,4-difluorophenyl)-4-methyl-2-oxo-1,6-dihydro-pyrimi
dine-5-carboxylic acid methyl ester (0.200 g, 0.500 mmol)
and (S)-(+)-3-methyl-1-(2-nitrophenyl)-piperazine (0.170
g, 0.750 mmol) in 20 mL of anhydrous acetone was added
powdered K₂CO₃ (0.34 g, 3.5 mmol) and KI (0.07 g, 0.5 mmol)
and the resulting suspension was heated at reflux
15 temperature for 10 h. TLC indicated a new spot for the
product (R_f = 0.3, 3/0.5 EtOAc/MeOH) and mostly the
starting material. The suspension was cooled, filtered
and the solvent was evaporated and the residue was
purified by column chromatography (EtOAc/MeOH, 5/1).
20 (+) - 1 , 2 , 3 , 6 -
Tetrahydro-1-{N-[2-(S)-methyl]-4-(2-nitrophenyl)piperazi
n - 1 - y 1] -
propyl}-carboxamido-4-methyl-6-(3,4-difluorophenyl)-
25 2-oxo-pyr-imidine was obtained as yellow oil (0.030 g, 10%
yield). The HCl salt was prepared by the addition of HCl
in ether to a solution of the product in dichloromethane,
followed by evaporation of the solvents; mp 150-153 °C;
[α]_D = 58.3 (c = 0.3, MeOH); ¹H NMR (CD₃OD) δ 1.04 (d, J=6.0
Hz, 3 H), 1.71-1.78 (m, 2 H), 2.33-2.49 (m, 3 H), 2.42 (s,
30 3 H), 2.55-2.92 (m, 5 H), 3.00-3.10 (m, 3 H), 3.34 -3.42
(m, 2 H), 3.72 (s, 3 H), 6.71 (s, 1 H), 7.01-7.32 (m, 6
H), 7.46 (dt, J=0.7 Hz, J=8.4 Hz, 1 H), 7.74 (dd, J=1.5,
8.4 Hz, 1 H), 8.82 (t, J=3.9 Hz, 1 H). Anal calcd. for
C₂₈H₃₃N₆F₂O₆ + 0.20 CH₂Cl₂ : C, 52.92; H, 5.26; N, 13.13.
35 Found: C, 52.84; H, 5.68; N, 12.94.

Example 35

1,2,3,6-TETRAHYDRO-1{N-[4-(2'-METHYL-PHENYL)PIPERAZIN-1-YL]COPROPYL}-CARBOXAMIDO-4-METHYL-6-(3,4-DIFLUOROPHENYL)-2-OXO-PYRIMIDINE: The amine used was 4-(2'-methyl-phenyl)-piperazine. ^1H NMR δ 1.75-1.80 (m, 2 H), 2.29 (s, 3 H), 2.42 (s, 3 H), 2.41-2.48 (m, 2 H), 2.58-2.62 (m, 4 H), 2.91-2.97 (m, 4 H), 3.35 -3.42 (m, 2 H), 3.72 (s, 3 H), 6.71 (s, 1 H), 6.97-7.26 (m, 8 H), 8.81 (t, $J=3.9$ Hz, 1 H). The product was dissolved in ether and 1 N HCl in ether was added. The ether was evaporated, giving the dihydrochloride salt; mp 66-71 °C. Anal calcd. for $\text{C}_{28}\text{H}_{35}\text{N}_5\text{F}_2\text{O}_4 \text{Cl}_2$ + 1.75 acetone: C, 55.73; H, 6.40; N, 9.78. Found: C, 56.16; H, 6.29; N, 10.06.

Example 36

(+)-1,2,3,6-TETRAHYDRO-5-METHOXCARBONYL-4-METHOXYMETHYL-2-OXO-1-{N-[3-(4-METHYL-4-PHENYL PIPERIDINE-1-YL]COPROPYL}-6-(3,4-DIFLUOROPHENYL) PYRIMIDINE: Hygroscopic; $[\alpha]_D = +82.1$ ($c = 0.31$, MeOH); ^1H NMR δ 1.14 (s, 3 H), 1.61-1.72 (m, 4 H), 2.03-2.08 (m, 2 H), 2.25 (t, $J=7.2$ Hz, 2 H), 2.30-2.42 (m, 4 H), 3.19-3.31 (m, 2 H), 3.40 (s, 3 H), 3.63 (s, 3 H), 4.60 (s, 2 H), 6.60 (s, 1 H), 6.97-7.29 (m, 8 H), 7.63 (br s, 1 H), 8.78 (t, $J=5.7$ Hz, 1 H). Anal calcd. for $\text{C}_{30}\text{H}_{37}\text{N}_4\text{O}_5\text{F}_2\text{Cl} + \text{CH}_2\text{Cl}_2$: C, 53.80; H, 5.68; N, 8.10. Found: C, 53.79; H, 6.03; N, 7.83.

EXAMPLE 37

5-(5-BUTYL-2-THIENYL) PYRIDO[2,3-d]PYRIMIDINE-2,4,7(1H,3H,8H)-

Example 38

METHYL (4S)-3-[{3-[4-(3-AMINOPHENYL)-1-
PIPERIDINYL]PROPYL}AMINO]CARBONYL]-4-(3,4-
DIFLUOROPHENYL)-6-(METHOXYMETHYL)-2-OXO-1,2,3,4-
TETRAHYDRO-5-PYRIMIDINECARBOXYLATE: ^1H NMR (400 MHz,
CDCl₃) δ 7.80 (s, 1H), 7.22-7.02 (m, 2H), 6.95 (t, 2H,
J=8.7 Hz), 6.63-6.44 (m, 4H), 4.56 (ABq, 2H), 3.62 (s,
3H), 3.33 (s, 3H), 3.32 (m, 4H), 2.96 (br s, 2H), 2.34
(t, 2H, J=7.5 Hz), 2.11-1.94 (m, 3H), 1.81-1.64 (m, 4H);
ESMS m/e: 572.3 (M + H)⁺.

Example 39

The product was obtained according to the method
described for Example 40.

METHYL (4S)-4-(3,4-DIFLUOROPHENYL)-3-{[3-(4-{3-
[(METHOXYACETYL)AMINO]PHENYL}-1-
PIPERIDINYL)PROPYL]AMINO}CARBONYL]-6-(METHOXYMETHYL)-2-
OXO-1,2,3,4-TETRAHYDRO-5-PYRIMIDINECARBOXYLATE: 15.6 mg
(69% yield); ^1H NMR (400 MHz, CDCl₃) δ 9.01 (s, 1H), 8.25
(s, 1H), 7.60 (s, 1H), 7.37 (d, 1H, J=7.2 Hz), 7.30-7.05
(m, 5H), 7.02 (d, 1H, J=8.0 Hz), 6.71 (s, 1H), 4.70 (s,
2H), 4.03 (s, 2H), 3.73 (s, 3H), 3.53 (s, 3H), 3.47 (s,
3H), 3.42-3.33 (m, 2H), 3.08 (br s, 2H), 2.49 (br s,
2H), 2.20 (s, 2H), 2.07 (br s, 1H), 1.97-1.75 (m, 4H);
ESMS m/e: 644.3 (M + H)⁺

Example 40

METHYL (4S)-4-(3,4-DIFLUOROPHENYL)-3-{[3-(4-{3-[
DIMETHYLBUTANOYL]AMINO]PHENYL}-1-
PIPERIDINYL)PROPYL]AMINO}CARBONYL]-6-(METHOXYMETHYL)-2-
OXO-1,2,3,4-TETRAHYDRO-5-PYRIMIDINECARBOXYLATE

To the 20 ml vial was added methyl (4S)-3-[({3-[4-(3-aminophenyl)-1-piperidinyl]propyl}amino)carbonyl]-4-(3,4-difluorophenyl)-6-(methoxymethyl)-2-oxo-1,2,3,4-tetrahydro-5-pyrimidinecarboxylate (0.035 mmol), an acid chloride or sulfonyl chloride (1.5 eq), N,N-diisopropylethylamine (5 eq) and dichloromethane (2 ml) at room temperature. The reaction mixture was stirred at room temperature for 24 h, at which time the TLC analysis indicated the reaction was completed. The reaction mixture was concentrated to a small volume and purified by preparative TLC (silica, 2000 microns, 95:5 = dichloromethane : methanol with 1% of isopropylamine) to give 5.6 mg of methyl (4S)-4-(3,4-difluorophenyl)-3-({[3-(4-{3-[(3,3-dimethylbutanoyl)amino]phenyl}-1-piperidinyl)propyl]amino}carbonyl)-6-(methoxymethyl)-2-oxo-1,2,3,4-tetrahydro-5-pyrimidinecarboxylate: 24.6% yield; ^1H NMR (400 MHz, CDCl_3) δ 7.50 (s, 1H), 7.26 (d, 1H, $J=8.3$ Hz), 7.15-7.02 (m, 5H), 6.88 (d, 1H, $J=8.3$ Hz), 6.55 (s, 1H), 4.56 (ABq, 2H), 3.62 (s, 3H), 3.32 (s, 3H), 3.25 (t, 4H, $J=9.0$ Hz), 2.99 (d, 2H, $J=10.8$ Hz), 2.49-2.37 (m, 3H), 2.08 (t, 2H, $J=11.7$ Hz), 1.78-1.65 (m, 14H); ESMS m/e: 670.4 ($\text{M} + \text{H}$)⁺.

Example 41

The product was obtained according to the method described for methyl (4S)-4-(3,4-difluorophenyl)-3-({[3-(4-{3-[(3,3-dimethylbutanoyl)amino]phenyl}-1-piperidinyl)propyl]amino}carbonyl)-6-(methoxymethyl)-2-oxo-1,2,3,4-tetrahydro-5-pyrimidinecarboxylate.

METHYL (4S)-4-(3,4-DIFLUOROPHENYL)-6-(METHOXYMETHYL)-2-OXO-3-{{[3-{4-[3-(PROPYNYLAMINO)PHENYL}-1-PIPERIDINYL]PROPYL]AMINO}CARBONYL}-1,2,3,4-TETRAHYDRO-5-

PYRIMIDINECARBOXYLATE: 9.9 mg (45% yield) δ ^1H NMR (400 MHz, CDCl_3) δ 7.36 (s, 1H), 7.28 (d, 1H, $J=8.0$ Hz), 7.16-7.02 (m, 5H), 6.86 (d, 1H, $J=7.6$ Hz), 6.54 (s, 1H), 4.56 (ABq, 2H), 3.62 (s, 3H), 3.32 (s, 3H), 3.27-3.19 (m, 4H), 2.95 (d, 2H, $J=10.3$ Hz), 2.41 (m, 1H), 2.34 (t, 2H, $J=7.7$ Hz), 2.28 (q, 2H, $J=7.6$ Hz), 2.01 (t, 2H, $J=11.1$ Hz), 1.73-1.64 (m, 8H); ESMS m/e: 628.4 ($M + H$)⁺

Example 42

The product was obtained according to the method described for methyl (4S)-4-(3,4-difluorophenyl)-3-({[3-(4-{3-[(3,3-dimethylbutanoyl)amino]phenyl}-1-piperidinyl)propyl]amino}carbonyl)-6-(methoxymethyl)-2-oxo-1,2,3,4-tetrahydro-5-pyrimidinecarboxylate.

METHYL (4S)-4-(3,4-DIFLUOROPHENYL)-6-(METHOXYMETHYL)-3-({[3-(4-{3-[(3-METHYLBUTANOYL)AMINO]PHENYL}-1-PIPERIDINYL)PROPYL]AMINO}CARBONYL)-2-OXO-1,2,3,4-TETRAHYDRO-5-PYRIMIDINECARBOXYLATE: 10.4 mg (45% yield)

δ ^1H NMR (400 MHz, CDCl_3) δ 7.36 (s, 1H), 7.28 (d, 1H, $J=7.9$ Hz), 7.16-7.03 (m, 5H), 6.88 (d, 1H, $J=7.4$ Hz), 6.56 (s, 1H), 4.56 (ABq, 2H), 3.62 (s, 3H), 3.32 (s, 3H), 3.25 (t, 4H, $J=6.7$ Hz), 2.98 (d, 2H, $J=11.1$ Hz), 2.43 (m, 1H), 2.38 (t, 2H, $J=7.5$ Hz), 1.13 (d, 2H, $J=7.5$ Hz), 2.10-2.01 (m, 2H), 1.75-1.64 (m, 6H), 0.91 (d, 6H, $J=5.8$ Hz); ESMS m/e: 656.4 ($M + H$)⁺

Example 43

The product was obtained according to the method described for methyl (4S)-4-(3,4-difluorophenyl)-3-({[3-(4-{3-[(3,3-dimethylbutanoyl)amino]phenyl}-1-piperidinyl)propyl]amino}carbonyl)-6-(methoxymethyl)-2-oxo-1,2,3,4-tetrahydro-5-pyrimidinecarboxylate.

METHYL (4S)-4-(3,4-DIFLUOROPHENYL)-3-{[(3-{4-[3-(ISOBUTYRYLAMINO)PHENYL]-1-PIPERIDINYL}PROPYL)AMINO]CARBONYL}-6-(METHOXYMETHYL)-2-OXO-1,2,3,4-TETRAHYDRO-5-PYRIMIDINECARBOXYLATE: 16.4 mg
(73% yield) δ 1 H NMR (400 MHz, CDCl₃) δ 7.37 (s, 1H), 7.28 (d, 1H, J=7.3 Hz), 7.16-7.01 (m, 5H), 6.88 (d, 2H, J=7.3 Hz), 6.54 (s, 1H), 4.56 (ABq, 2H), 3.62 (s, 3H), 3.32 (s, 3H), 3.25 (t, 2H, J=6.8 Hz), 3.23-3.18 (m, 2H), 3.03 (d, 2H, J=11.7 Hz), 2.57-2.48 (m, 1H), 2.43 (t, 2H, J=8.0 Hz), 2.14 (t, 2H, J=9.4 Hz), 1.8-1.65 (m, 5H), 1.09 (d, 6H, J=6.3 Hz); ESMS m/e: 642.4 (M + H)⁺

Example 44

The product was obtained according to the method described for methyl (4S)-4-(3,4-difluorophenyl)-3-{[(3-{4-[3,3-dimethylbutanoyl]amino}phenyl)-1-piperidinyl]propyl]amino}carbonyl)-6-(methoxymethyl)-2-oxo-1,2,3,4-tetrahydro-5-pyrimidinecarboxylate.

METHYL (4S)-3-{[(3-{4-[3-(BUTYRYLAMINO)PHENYL]-1-PIPERIDINYL}PROPYL)AMINO]CARBONYL}-4-(3,4-DIFLUOROPHENYL)-6-(METHOXYMETHYL)-2-OXO-1,2,3,4-TETRAHYDRO-5-PYRIMIDINECARBOXYLATE: 14.7 mg (65.5% yield) δ 1 H NMR (400 MHz, CDCl₃) δ 7.38 (s, 1H), 7.26 (s, 1H), 7.17-6.99 (m, 5H), 6.87 (s, 1H), 6.55 (s, 1H), 4.56 (ABq, 2H), 3.63 (s, 3H), 3.33 (s, 3H), 3.28-3.17 (m, 6H), 3.0 (br s, 2H), 2.51-2.36 (m, 3H), 2.25 (t, 2H, J=5.0 Hz), 2.10 (br s, 2H), 1.8-1.56 (m, 6H), 0.90 (t, 3H, J=5.0 Hz); ESMS m/e: 642.4 (M + H)⁺.

Example 45

(4R)-N-(3-{4-[3-(BUTYRYLAMINO)PHENYL]-1-PIPERIDINYL}PROPYL)-4-(3,4-DIFLUOROPHENYL)-6-(METHOXYMETHYL)-2-OXO-1,2,3,4-TETRAHYDRO-5-PYRIMIDINECARBOXAMIDE

5

Method:

(4R)-4-(3,4-difluorophenyl)-6-(methoxymethyl)-2-oxo-1,2,3,4-tetrahydro-5-pyrimidinecarboxylic acid: A

10 stirred mixture of one mole equivalent of methyl (4R)-4-(3,4-difluorophenyl)-6-(methoxymethyl)-2-oxo-1,2,3,4-tetrahydro-5-pyrimidinecarboxylate (10.0 g, 32.0 mmol) and lithium hydroxide (2 equivalents, 1.53 g, 64.0 mol) in H₂O-THF (2:1, 300 mL) was heated at reflux temperature
15 for 1 h. The reaction mixture was concentrated, dissolved in water, washed with ethyl acetate and acidified (1 N HCl) to pH 3-4 (pH paper). The precipitated product was collected, washed with water and dried under reduced pressure to give the desired
20 product in 90% yield.

(4R)-4-(3,4-DIFLUOROPHENYL)-6-(METHOXYMETHYL)-N-[3-(4-(3-NITROPHENYL)-3,6-DIHYDRO-1(2H)-PYRIDINYL)PROPYL]-2-OXO-1,2,3,4-TETRAHYDRO-5-PYRIMIDINECARBOXAMIDE: A

25 solution of (4R)-4-(3,4-difluorophenyl)-6-(methoxymethyl)-2-oxo-1,2,3,4-tetrahydro-5-pyrimidinecarboxylic acid (1.2 eq), EDC (1.5 Eq.), N-methylmorpholine (2.0 Eq.) in dichloromethane was stirred at room temperature for 15 minutes, followed by
30 addition of 3-(4-(3-nitrophenyl)-3,6-dihydro-1(2H)-pyridinyl)-1-propanamine (1.0 eq.) to the reaction mixture. The resulting solution was stirred for 18 hours, concentrated and chromatographed on silica to

give (*4R*) -4-(3,4-difluorophenyl)-6-(methoxymethyl)-*N*-[3-(4-(3-nitrophenyl)-3,6-dihydro-1(2*H*)-pyridinyl)propyl]-2-oxo-1,2,3,4-tetrahydro-5-pyrimidinecarboxamide.

- 5 **(4R)-*N*-{3-[4-(3-AMINOPHENYL)-1-PIPERIDINYL]PROPYL}-4-(3,4-DIFLUOROPHENYL)-6-(METHOXYMETHYL)-2-OXO-1,2,3,4-TETRAHYDRO-5-PYRIMIDINECARBOXAMIDE:** A mixture of (*4R*)-4-(3,4-difluorophenyl)-6-(methoxymethyl)-*N*-[3-(4-(3-nitrophenyl)-3,6-dihydro-1(2*H*)-pyridinyl)propyl]-2-oxo-1,2,3,4-tetrahydro-5-pyrimidinecarboxamide, 10% Pd/C in ethanol was hydrogenated (balloon method) for 2 days. The reaction mixture was filtered through Celite 545, washed with ethanol and concentrated to give the desired product.
- 10 15 **(4R)-*N*-(3-{4-[3-(BUTYRYLAMINO)PHENYL]-1-PIPERIDINYL}PROPYL)-4-(3,4-DIFLUOROPHENYL)-6-(METHOXYMETHYL)-2-OXO-1,2,3,4-TETRAHYDRO-5-PYRIMIDINECARBOXAMIDE:** Into a 20 mL vial was added (*4R*)-*N*-{3-[4-(3-aminophenyl)-1-piperidinyl]propyl}-4-(3,4-difluorophenyl)-6-(methoxymethyl)-2-oxo-1,2,3,4-tetrahydro-5-pyrimidinecarboxamide (0.040 mmol), acid chloride (1.5 eq) and N,N-diisopropylethylamine (5.0 eq) in 2.0 mL of dichloromethane at room temperature. After 24 hrs, the reaction mixture was concentrated *in vacuo* and purified by preparative TLC (silica, 2000 microns, 95:5 = dichloromethane : methanol with 1% of isopropylamine) to give 9.2 mg (45% yield) of the desired product: ^1H NMR (400 MHz, CD₃OD) δ 7.49 (s, 1H), 7.25 (d, 1H, J=7.6 Hz), 7.20-7.02 (m, 5H), 6.91 (d, 1H, J=8 Hz), 5.29 (s, 1H), 4.24 (ABq, 2H), 3.30 and 3.24 (two s, 3H), 3.46-3.12 (m, partially hidden by three s, 4H), 2.74 (br s, 4H), 2.25 (t, 2H, J=8.2 Hz), 2.04-1.69
- 20 25 30

(m, 7H), 1.63 (sextet, 2H, J=7.4 Hz), 0.91 (t, 3H, 7.4 Hz); ESMS m/e: 584.4 (M + H)⁺.

Example 46

5 The product was obtained according to the method described for (4R)-N-(3-{4-[3-(butyrylamino)phenyl]-1-piperidinyl}propyl)-4-(3,4-difluorophenyl)-6-(methoxymethyl)-2-oxo-1,2,3,4-tetrahydro-5-pyrimidinecarboxamide.

10 (4R)-4-(3,4-DIFLUOROPHENYL)-6-(METHOXYMETHYL)-2-OXO-N-(3-{4-[3-(PROPYNYLAMINO)PHENYL]-1-PIPERIDINYL}PROPYL)-1,2,3,4-TETRAHYDRO-5-PYRIMIDINECARBOXAMIDE: 5.6 mg
(24.6% yield); ¹H NMR (400 MHz, CD₃OD) δ 7.56 (s, 1H), 7.35 (d, 1H, J=6.9 Hz), 7.3-7.03 (m, 4H), 7.17 (br s, 1H), 6.99 (d, 1H, J=7.0 Hz), 5.45 (s, 1H), 4.33 (ABq, 2H), 3.41 (s, 3H), 3.37-3.23 (m, partially hidden, 4H), 2.8 (br s, 4H), 2.39 (d, 2H, J=9.3 Hz), 2.14-1.78 (m, 7H), 1.21 (t, 3H, J=7.6 Hz); ESMS m/e: 570.4 (M + H)⁺.

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Example 47

The product was obtained according to the method described for (4R)-N-(3-{4-[3-(butyrylamino)phenyl]-1-piperidinyl}propyl)-4-(3,4-difluorophenyl)-6-(methoxymethyl)-2-oxo-1,2,3,4-tetrahydro-5-pyrimidinecarboxamide.

20 (4R)-4-(3,4-DIFLUOROPHENYL)-6-(METHOXYMETHYL)-N-[3-(4-{3-[3-METHYLBUTANOYL]AMINO}PHENYL)-1-PIPERIDINYL]PROPYL]-2-OXO-1,2,3,4-TETRAHYDRO-5-PYRIMIDINECARBOXAMIDE: 11.1 mg (46% yield); ¹H NMR (400 MHz, CD₃OD) δ 7.81 (d, 1H, J=8.5 Hz), 7.6 (s, 1H), 7.55 (s, 1H), 7.36 (br s, 1H), 7.31-7.17 (m, 3H), 7.01 (t, 1H,

J=6.7 Hz) 6.64-6.61 (m, 1H), 5.45 (br s, 1H), 4.32 (ABq, 2H), 3.94 and 3.87 (two s, 3H), 3.42-3.12 (m, partially hidden, 2H), 3.1 (br s, 2H), 3.0 (t, 2H, J=11.1 Hz), 2.79-2.57 (m, 4H), 2.27-1.73 (m, 8H), 1.19 and 1.01 (two d, 6H, J=6.6 Hz); ESMS m/e: 598.4 (M + H)⁺.

Example 48

The product was obtained according to the method described for (4R)-N-(3-{4-[3-(butyrylamino)phenyl]-1-piperidinyl}propyl)-4-(3,4-difluorophenyl)-6-(methoxymethyl)-2-oxo-1,2,3,4-tetrahydro-5-pyrimidinecarboxamide.

(4R)-4-(3,4-DIFLUOROPHENYL)-6-(METHOXYMETHYL)-N-[3-(4-{3-[(2-METHYLBUTANOYL)AMINO]PHENYL}-1-PIPERIDINYL)PROPYL]-2-OXO-1,2,3,4-TETRAHYDRO-5-PYRIMIDINECARBOXAMIDE: 6.7 mg (28% yield); ¹H NMR (400 MHz, CD₃OD) δ 7.59 (s, 1H), 7.35 (br s, 1H), 7.3-7.2 (m, 3H), 7.17 (br s, 1H), 7.01 (d, 1H, J=6.8 Hz), 5.45 (s, 1H), 4.33 (ABq, 2H), 3.39 (s, 3H), 3.29 (m, 2H), 2.84 (br s, 4H), 2.42 (m, 1H), 2.14-1.78 (m, 9H), 1.7 (m, 1H), 1.49 (m, 1H), 1.20 (d, 3H, J=6.7 Hz), 0.95 (t, 3H, J=6.6 Hz); ESMS m/e: 598.4 (M + H)⁺.

Example 49

The product was obtained according to the method described for (4R)-N-(3-{4-[3-(butyrylamino)phenyl]-1-piperidinyl}propyl)-4-(3,4-difluorophenyl)-6-(methoxymethyl)-2-oxo-1,2,3,4-tetrahydro-5-pyrimidinecarboxamide.

(4R)-4-(3,4-DIFLUOROPHENYL)-N-[3-(4-{3-[(3,3-DIMETHYLBUTANOYL)AMINO]PHENYL}-1-PIPERIDINYL)PROPYL]-6-

(METHOXYMETHYL) -2-OXO-1,2,3,4-TETRAHYDRO-5-PYRIMIDINECARBOXAMIDE: 1.1 mg (4.4% yield); ^1H NMR (400 MHz, CD_3OD) δ 7.6-6.91 (m, 7H), 5.43 (s, 1H), 4.31 (ABq, 2H), 3.40 (s, 3H), 3.27-1.26 (m, 17 H), 1.09 (s, 9H);
5 ESMS m/e: 612.4 ($M + H$)⁺.

Example 50

The product was obtained according to the method described for (4R)-N-(3-{4-[3-(butyrylamino)phenyl]-1-piperidinyl}propyl)-4-(3,4-difluorophenyl)-6-(methoxymethyl)-2-oxo-1,2,3,4-tetrahydro-5-pyrimidinecarboxamide.

(4R)-4-(3,4-DIFLUOROPHENYL)-N-(3-{4-[3-(ISOBUTYRYLAMINO) PHENYL]-1-PIPERIDINYL}PROPYL)-6-(METHOXYMETHYL)-2-OXO-1,2,3,4-TETRAHYDRO-5-PYRIMIDINECARBOXAMIDE: 12.7 mg (54% yield); ^1H NMR (400 MHz, CD_3OD) δ 7.59 (s, 1H), 7.36 (d, 1H, $J=8.6$ Hz), 7.31-7.07 (m, 4H), 7.01 (d, 1H, $J=6.5$ Hz), 5.39 (s, 1H), 4.34 (ABq, 2H), 3.35 (s, 3H), 3.33-3.19 (m, partially hidden, 2H), 3.08-2.72 (m, 4H), 2.63 (t, 2H, $J=7.2$ Hz), 2.14-1.82 (m, 8H), 1.19 (d, 6H, $J=6.9$ Hz); ESMS m/e: 584.4 ($M + H$)⁺.

25 **Example 51**

The synthetic method is the same as described for the synthesis of (4S)-N-(3-{4-[3-(acetylamino)phenyl]-1-piperidinyl}propyl)-4-(3,5-difluorophenyl)-2-oxo-1,3-oxazolidine-3-carboxamide.

30 5-ACETYL-N-(3-{4-[3-(ACETYLAMINO) PHENYL]-1-PIPERIDINYL}PROPYL)-4-METHYL-2-OXO-6-(3,4,5-TRIFLUOROPHENYL)-3,6-DIHYDRO-1(2H)-

PYRIMIDINECARBOXAMIDE: 14.5 mg (46% yield); ^1H NMR (400 MHz, CDCl_3) δ 9.56 (s, 1H), 9.20 (s, 1H), 8.21 (s, 1H), 7.52 (s, 1H), 7.18 (t, 1H, $J=7.8$ Hz), 7.07-6.75 (m, 5H), 3.59-3.37 (m, 1H), 3.48-3.38 (m, 1H), 3.08 (br s, 2H), 2.57-2.39 (m, 5H), 2.25 (s, 3H), 2.21 (s, 3H), 2.19-1.59 (m, 9H); ESMS m/e: 586.3 ($M + H$) $^+$; Anal. Calc. for $\text{C}_{30}\text{H}_{34}\text{F}_3\text{N}_5\text{O}_4 + 0.1\text{CHCl}_3$: C, 60.50; H, 5.75; N, 11.72. Found: C, 60.59; H, 5.40; N, 11.73.

10 **Example 52**

The synthetic method is the same as described for the synthesis of (4S)-N-(3-{4-[3-(acetylamino)phenyl]-1-piperidinyl}propyl)-4-(3,5-difluorophenyl)-2-oxo-1,3-oxazolidine-3-carboxamide.

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BENZYL 3-{[(3-{4-[3-(ACETYLAMINO) PHENYL]-1-PIPERIDINYL}PROPYL)AMINO]CARBONYL}-4-(2,4-DIFLUOROPHENYL)-6-ETHYL-2-OXO-1,2,3,4-TETRAHYDRO-5-PYRIMIDINECARBOXYLATE: 14.8 mg (41% yield); ^1H NMR (400

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MHz, CDCl_3) δ 9.05 (br s, 1H), 8.14 (s, 1H), 7.47 (s, 1H), 7.37-7.21 (m, 8H), 7.18 (t, 1H, $J=7.7$ Hz), 6.94 (d, 1H, $J=6.9$ Hz), 6.87 (d, 1H, $J=7.4$ Hz), 6.7-6.62 (m, 3H), 5.09 (q, 2H, $J=17.8$ Hz), 3.48-3.24 (m, 2H), 3.04 (ABq, 2H), 2.88-2.71 (m, 2H), 2.52-2.39 (m, 2H), 2.19 (s, 3H), 2.17-1.88 (m, 3H), 1.77-1.58 (m, 3H), 1.19 (t, 3H, $J=7.5$ Hz); ESMS m/e: 674.4 ($M + H$) $^+$.

25 **Example 53**

The synthetic method is the same as described for the synthesis of (4S)-N-(3-{4-[3-(acetylamino)phenyl]-1-piperidinyl}propyl)-4-(3,5-difluorophenyl)-2-oxo-1,3-oxazolidine-3-carboxamide.

5

N-(3-{4-[3-(ACETYLAMINO) PHENYL]-1-PIPERIDINYL}PROPYL)-4-(1,3-BENZODIOXOL-5-YL)-2,5-DIOXO-1,2,5,7-TETRAHYDROFURO[3,4-D] PYRIMIDINE-3(4H)-CARBOXAMIDE: 8.75 mg (28% yield); ^1H NMR (400 MHz, CDCl_3) δ 9.81 (s, 1H), 8.14 (s, 1H), 7.53 (s, 1H). 7.21 (t, 1H, $J=7.7$ Hz), 6.99 (d, 1H, $J=7.7$ Hz), 6.91-6.7 (m, 4H), 6.42 (s, 1H), 5.9 (s, 2H), 4.75 (s, 2H), 3.61-3.5 (m, 1H), 3.37-3.27 (m, 1H), 3.08 (br s, 2H), 2.56-2.40 (m, 3H), 2.18 (s, 3H), 2.16-1.85 (m, 4H), 1.78-1.6 (m, 5H); ESMS m/e: 576.3 (M + H) $^+$.

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Example 54

The synthetic method is the same as described for the synthesis of (4S)-N-(3-{4-[3-(acetylamino)phenyl]-1-piperidinyl}propyl)-4-(3,5-difluorophenyl)-2-oxo-1,3-oxazolidine-3-carboxamide.

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30

METHYL 1-{[(3-{4-[3-(ACETYLAMINO) PHENYL]-1-PIPERIDINYL}PROPYL)AMINO]CARBONYL}-2-[(4-METHOXYBENZYL)SULFANYL]-4-METHYL-6-(4-NITROPHENYL)-1,6-DIHYDRO-5-PYRIMIDINECARBOXYLATE: 10.1 mg (26% yield); ^1H NMR (400 MHz, CDCl_3) δ 8.02 (d, 2H, $J=7.5$ Hz), 7.53 (br s, 1H), 7.44-7.27 (m, 6H), 7.14 (d, 2H, $J=8.5$ Hz), 6.99 (d, 1H, $J=7.6$ Hz), 6.75 (d, 2H, $J=8.5$ Hz), 6.2 (s, 1H), 4.23 (ABq, 2H), 3.78 (s, 3H), 3.7 (s, 3H), 3.58-3.48 (m, 1H), 3.37-3.26 (m, 2H), 3.04 (m, 2H), 2.61-2.43 (m, 3H), 2.41 (s, 3H), 2.16 (s, 3H), 2.15-1.64 (m, 8H); ESMS m/e: 729.3 (M + H) $^+$.

Example 55

The synthetic method is the same as described for the synthesis of (4S)-N-(3-{4-[3-(acetylamino)phenyl]-1-piperidinyl}propyl)-4-(3,5-difluorophenyl)-2-oxo-1,3-oxazolidine-3-carboxamide.

N- (3- {4- [3- (ACETYLAMINO) PHENYL] -1-PIPERIDINYL}PROPYL) -4-
10 (2,1,3-BENZOXADIAZOL-5-YL) -2,5-DIOXO-1,2,5,7-
TETRAHYDROFURO [3,4-D] PYRIMIDINE-3 (4H) -CARBOXAMIDE: 7.7
mg (12% yield); ^1H NMR (400 MHz, CDCl_3) δ 7.97-6.83 (m,
7H), 6.49 (s, 1H), 5.51(s, 1H), 3.43-2.02 (m, 17 H),
1.82 (s, 3H); ESMS m/e: 574.3 ($\text{M} + \text{H}$)⁺.

15 **Example 56**

The synthetic method is the same as described for the synthesis of (4S)-N-(3-{4-[3-(acetylamino)phenyl]-1-piperidinyl}propyl)-4-(3,5-difluorophenyl)-2-oxo-1,3-oxazolidine-3-carboxamide.

20 METHYL (4S)-3- { [(3-{4-[3-(ACETYLAMINO) PHENYL]-1-
PIPERIDINYL}PROPYL)AMINO]CARBONYL} -4-(3,4-
DIFLUOROPHENYL) -6-METHYL-2-OXO-1,2,3,4-TETRAHYDRO-5-
PYRIMIDINECARBOXYLATE: 16.6 mg (52% yield); ^1H NMR (400
MHz, CDCl_3) δ 9.55 (br s, 1H), 9.07 (s, 1H), 8.19 (s,
1H), 7.54 (s, 1H), 7.25-6.98 (m, 4H), 6.95 (d, 1H, $J=8.0$
Hz), 6.81 (d, 1H, $J=7.5$ Hz), 6.69 (s, 1H), 3.70 (s, 3H),
3.57-3.34 (m, 2H), 3.06 (t, 2H, $J=11.6$ Hz), 2.47 (t, 2H,
 $J=8.1$ Hz), 2.42 (s, 3H), 2.20 (s, 3H), 2.18-1.61 (m,
9H); ESMS m/e: 584.3 ($\text{M} + \text{H}$)⁺; Anal. Calc. for
30 $\text{C}_{30}\text{H}_{35}\text{F}_2\text{N}_5\text{O} + 0.25\text{CHCl}_3$: C, 59.23; H, 5.79; N, 11.42. Found:
C, 59.61; H, 5.31; N, 11.48.

Peptide Synthesis:

Abbreviations: Fmoc: 9-Fluorenyloxycarbonyl-; Trityl:
triphenylmethyl-; tBu-: tertiary butyl ester; OtBu-:
tertiary butyl ether; Ng: N-guanidinyl; Nin: N-Indole;
MBHA : methylbenzhydrolamine; DMF: N,N-dimethylformamide;
NMP: N-Methylpyrrolidinone; DIEA: diisopropylethyl amine;
TFA: trifluoroacetic acid.

- Small scale peptide syntheses were performed either manually, by using a sintered glass column with argon pressure to remove solvents and reagents, or by using an Advanced ChemTech 396-9000 automated peptide synthesizer (Advanced ChemTech, Louisville, KY). Large scale peptide syntheses were performed on a CS Bio 536 (CS Bio Inc., San Carlos, CA). Fmoc-Alanine-OH, Fmoc-Cysteine(Trityl)-OH, Fmoc-Aspartic acid(tBu)-OH, Fmoc-Glutamic acid(tBu)-OH, Fmoc-Phenylalanine-OH, Fmoc-Glycine-OH, Fmoc-Histidine(Trityl)-OH, Fmoc-Isoleucine-OH, Fmoc-Lysine(Boc)-OH, Fmoc-Leucine-OH, Fmoc-Methionine-OH, Fmoc-Asparagine(Trityl)-OH, Fmoc-Proline-OH, Fmoc-Glutamine(Trityl)-OH, Fmoc-Arginine(Ng-2,2,4,6,7-Pentamethyldihydrobenzofuran-5-sulfonyl)-OH, Fmoc-Serine(OtBu-OH), Fmoc-Threonine(OtBu)-OH, Fmoc-Valine-OH, Fmoc-Tryptophan(NinBoc)-OH, Fmoc-Tyrosine(OtBu)-OH, Fmoc-Cyclohexylalanine-OH, and Fmoc-Norleucine, Fmoc -O-benzyl-phosphotyrosine were used as protected amino acids. Any corresponding D-amino acids had the same side-chain protecting groups, with the exception of Fmoc-D-Arginine, which had a Ng-2,2,5,7,8-pentamethylchroman-6-sulfonyl protecting group.
- Peptides with C-terminal amides were synthesized on solid phase using Rink amide-MBHA resin. The Fmoc group of the Rink Amide MBHA resin was removed by treatment with 30%

piperidine in DMF for 5 and 30 minutes respectively. After washing with DMF (3 times), methanol (2 times) and DMF/NMP (3 times), the appropriate Fmoc-protected amino acid (4 eq.) was coupled for 2 hours with HBTU or HATU (4eq.) as the activating agent and DIEA (8eq.) as the base. In manual syntheses, the ninhydrin test was used to test for complete coupling of the amino acids. The Fmoc groups were removed by treatment with 30% piperidine in DMF for 5 and 30 minutes respectively. After washing with DMF (3 times), methanol (2 times) and DMF/NMP (3 times), the next Fmoc-protected amino acid (4 eq.) was coupled for 2 hours with HBTU or HATU (4eq.) as the activating agent and DIEA (8eq.) as the base. This process of coupling and deprotection of the Fmoc group was continued until the desired peptide was assembled on the resin. The N-terminal Fmoc group was removed by treatment with 30% piperidine in DMF for 5 and 30 minutes respectively. After washing with DMF (3 times), methanol (2 times), the resin(s) was vacuum dried for 2 hours. Cleavage of the peptide-on-resin and removal of the side chain protecting groups was achieved by treating with TFA : ethanedithiol : thioanisole: m-cresol : water : triisopropylsilane : phenol, 78/5/3/3/3/5/3 (5 mL per 100 mg resin) for 2.5-3 hours. The cleavage cocktail containing the peptide was filtered into a round bottom flask and the volatile liquids were removed by rotary evaporation at 30-40 °C. The peptides were precipitated with anhydrous ether, collected on a medium-pore sintered glass funnel by vacuum filtration, washed with ether and vacuum dried.

Peptides with C-terminal acids were synthesized using 2-chlorotrityl chloride resin. The first amino acid was attached to the resin by dissolving 0.6-1.2eq. of the appropriate Fmoc-protected amino acid described above in dichloromethane (a minimal amount of DMF was added to

facilitate the dissolution, if necessary). To this was added DIEA (4 eq. Relative to the Fmoc-amino acid) and the solution was added to the resin and shaken for 30-120 minutes. The solvents and the excess reagents were
5 drained and the resin was washed with dichloromethane / methanol / DIEA (17/2/1) (3 times), dichloromethane (3 times), DMF (2 times), dichloromethane (2 times), and vacuum dried. The process of deprotection of the Fmoc group and coupling the appropriate Fmoc-protected amino
10 acid was continued as described above, until the desired, fully protected peptide was assembled on the resin. The process for removal of the final Fmoc group and the cleavage and deprotection of the peptides was the same as described above for the peptides with C-terminal amides.

15

Purification of the peptides was achieved by preparative high performance column chromatography (HPLC), using a reverse-phase C-18 column (25 x 250mm) (Primesphere or
20 Vydac) with a gradient of acetonitrile (0.1% TFA) in water (0.1% TFA). The general gradient was from 10%-90% acetonitrile in water over 40 minutes. The fractions corresponding to each peak on the HPLC trace was collected, freeze dried and analyzed by electrospray mass
25 spectrometry. The fraction having the correct mass spectral data corresponding to the desired peptide was then further analyzed by amino acid analysis, if necessary. All purified peptides were tested for homogeneity by analytical HPLC using conditions similar to
30 that described above, but by using a 2.5x250 mm analytical column, and generally were found to have >95% purity.

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35 See our published dihydropyrimidinone and oxazolidinone

patents as references for the synthesis of the templates and the piperidines.

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ISSN:0022-2623. CAN 130:110137 AN 1998:742998 CAPLUS

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For the general procedure for Pd coupling of vinyl triflate and boronic acids or tributyl tin reagents: See, Wuston, Wise *Synthesis* 1991, 993)

10

(For Typical References, See: Schroeter, G. *Ber.* (1909) 42, 3356; and Allen, C.F.H.; Bell, A. *Org. Syn. Coll. Vol. 3,* (1955) 846).

15

For the preparation of the ether N-[4-(benzo-4',5' [H]-furanpiperidine refer to W.E.Parham et al, *J. Org. Chem.* (1976) 41, 2268.

20

For the preparation of the ether piperidine precursor of Example 20, refer to W.E.Parham et al, *J. Org. Chem.* (1976) 41, 2268.

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For the preparation of the indane piperidine precursor of Example 21, refer to M.S.Chambers *J. Med. Chem.* (1992) 35, 2033.

30

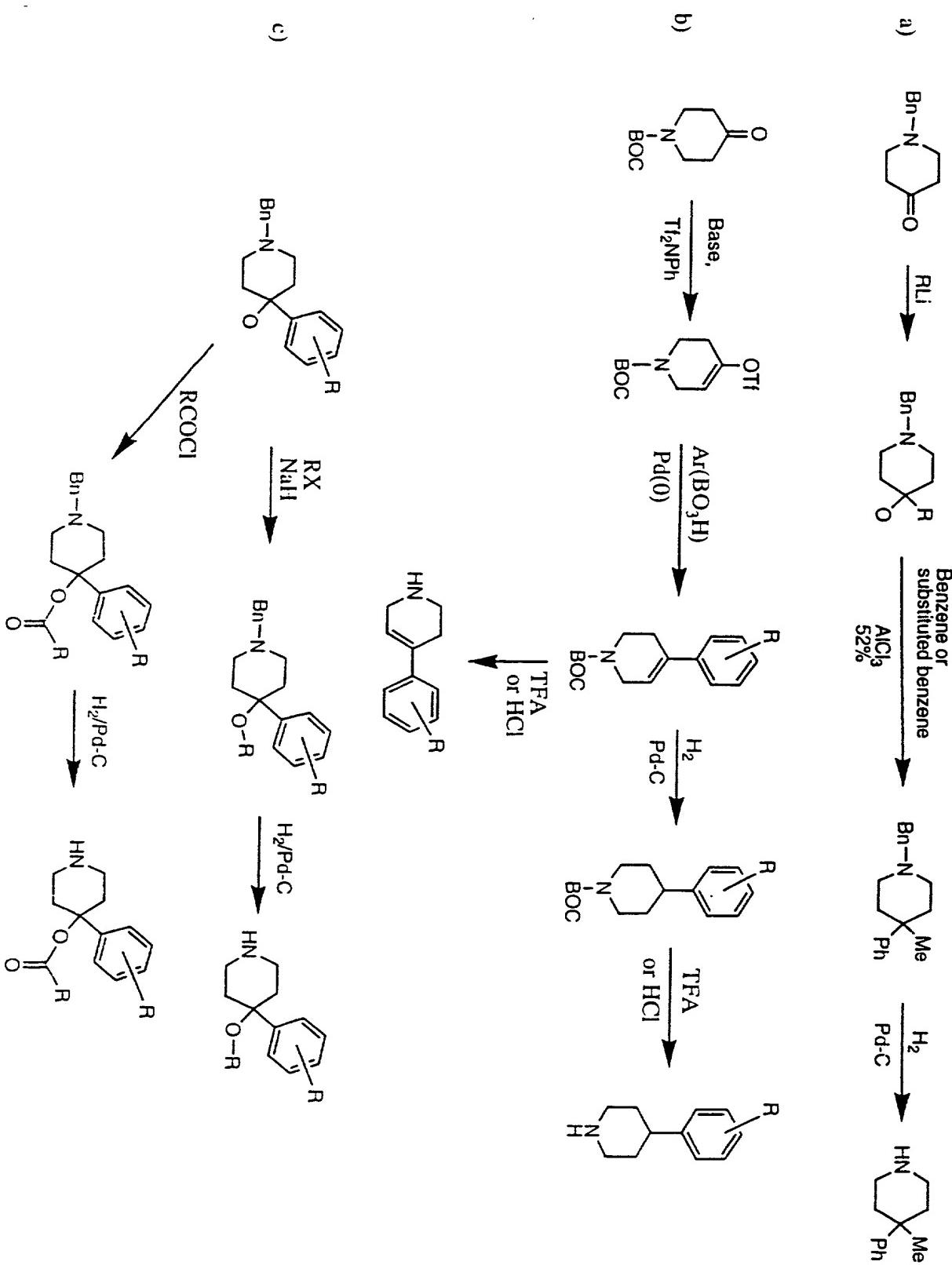
For the preparation of the piperidine precursor of Example 23, (K.Hashigaki et al. *Chem.Pharm.Bull.* (1984) 32, 3568.)

35

For the preparation of the piperidine precursor of Example 32, spiro[1H-indane-1,4'-piperidine], refer to M.S.Chambers et al. *J. Med. Chem.* (1992) 35, 2033.)

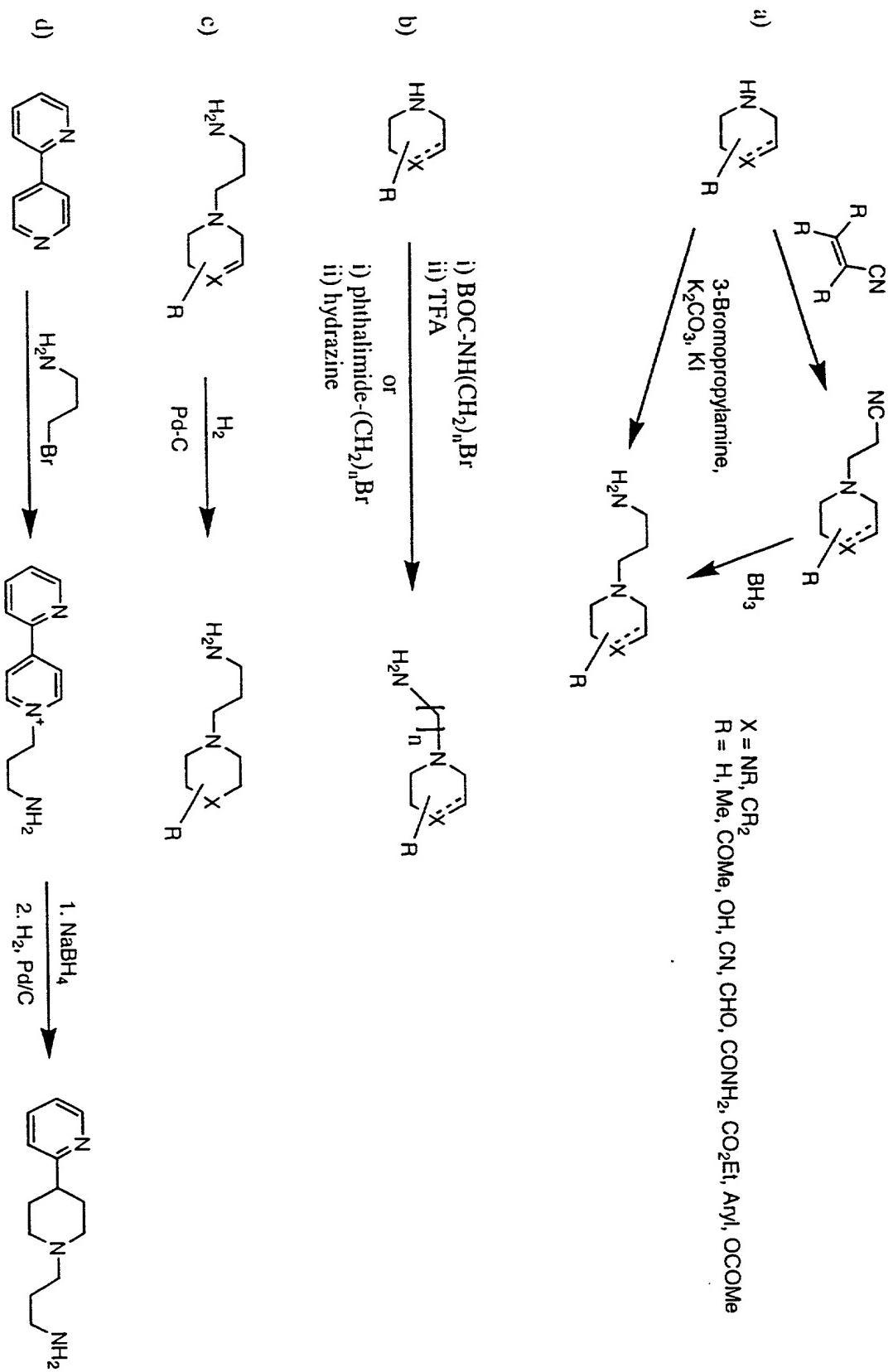
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Scheme 1. Synthesis of Precursor Compounds

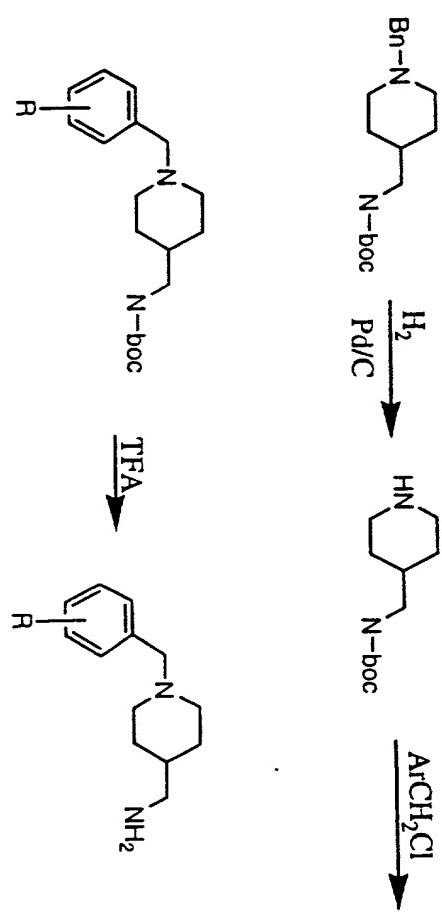


Scheme 2. Synthesis of Precursor Compounds

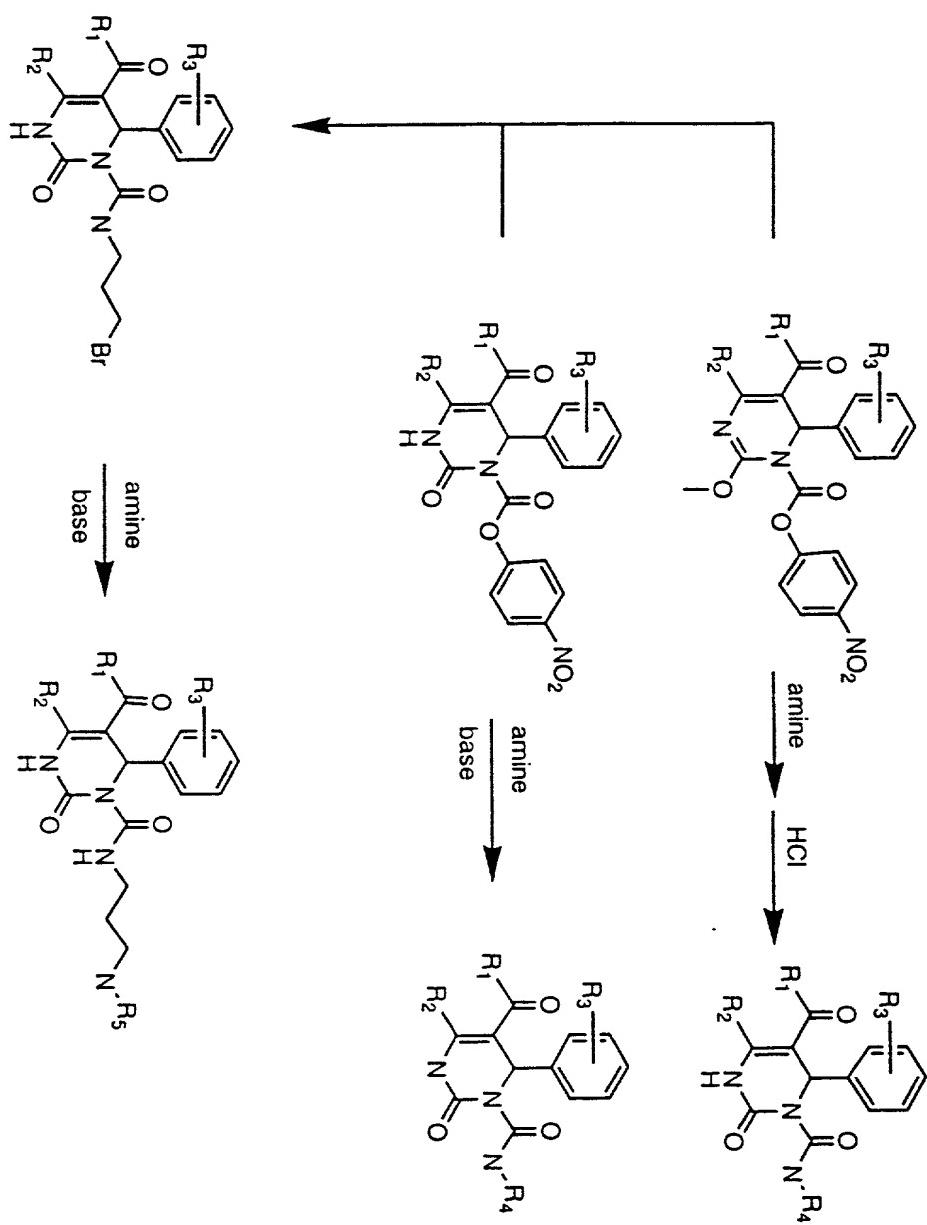
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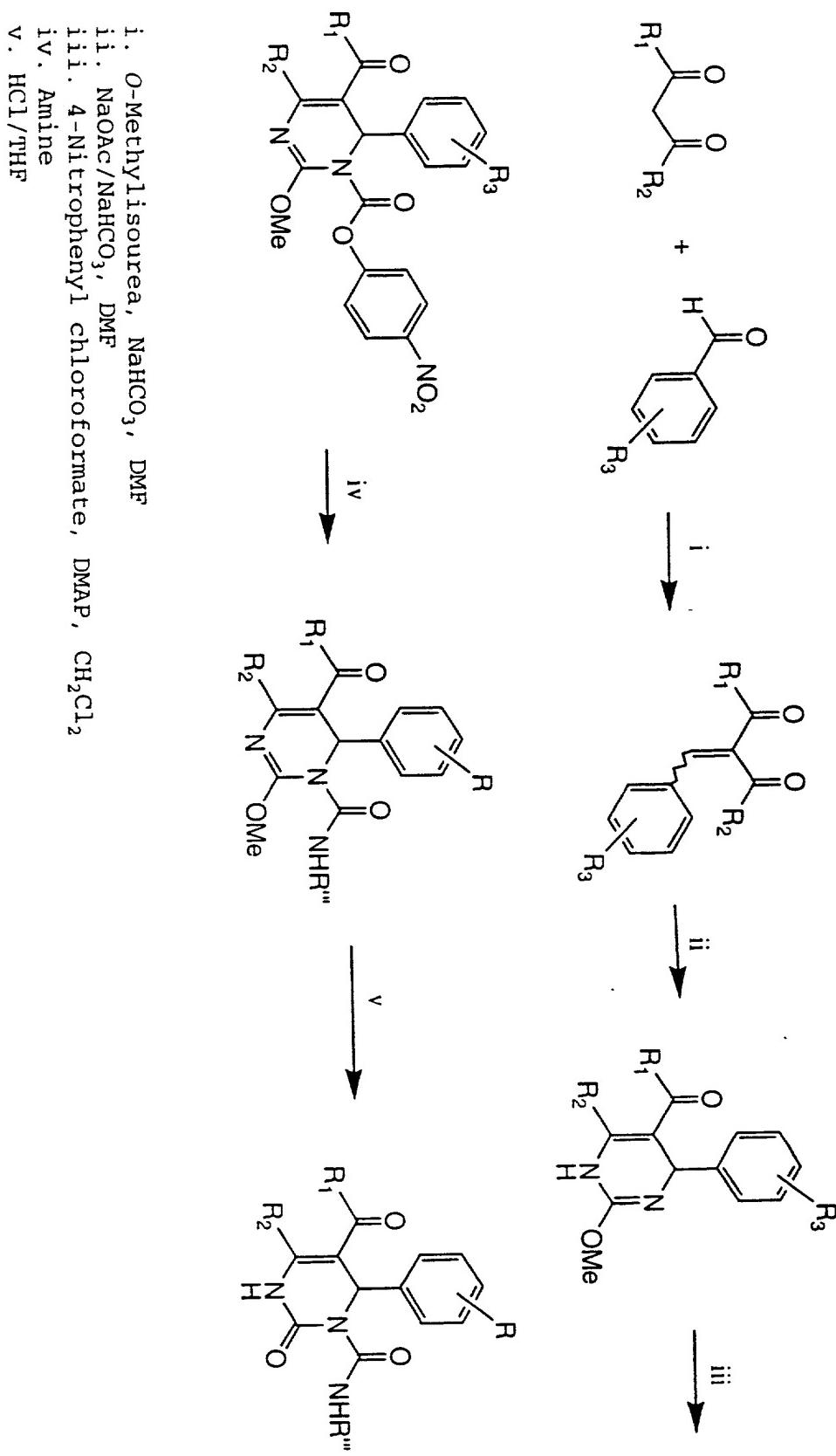
Scheme 3. Synthesis of Precursor Compounds



Scheme 4. Synthesis of Various Dihydropyrimidinones



Scheme 5. Synthesis of Dihydropyrimidinones



i. O-Methylisourea, NaHCO₃, DMF

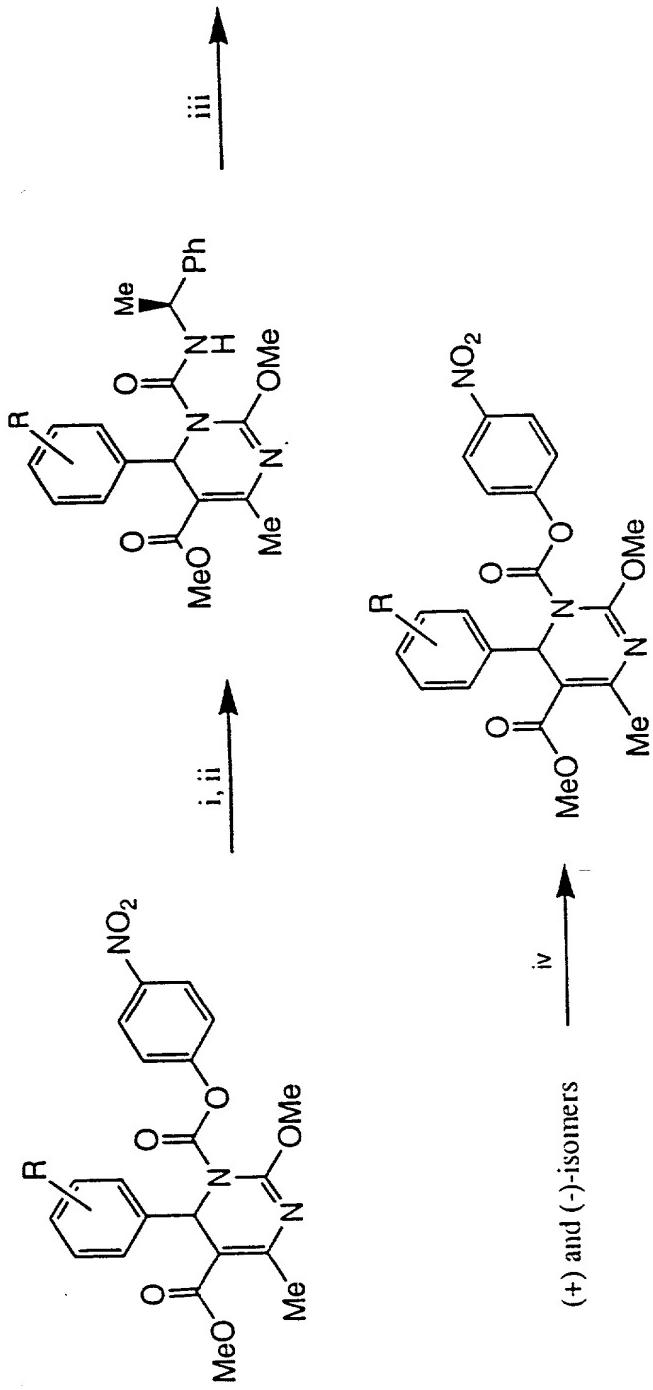
ii. NaOAc/NaHCO₃, DMF

iii. 4-Nitrophenyl chloroformate, DMAP, CH₂Cl₂

iv. Amine

v. HCl/THF

Scheme 6. Resolution of dihydropyrimidinones.



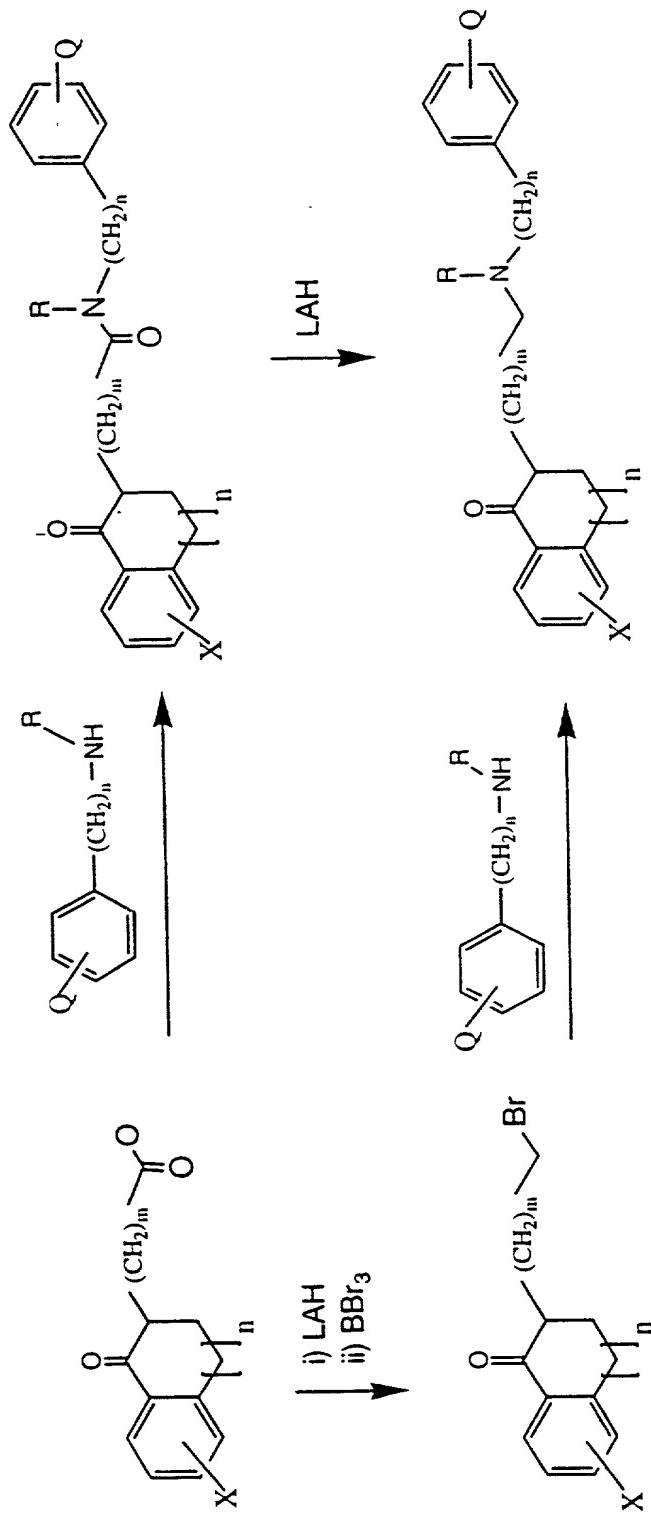
i. S-($-$)- α -Methylbenzylamine

ii. Sepn. of diastereomers

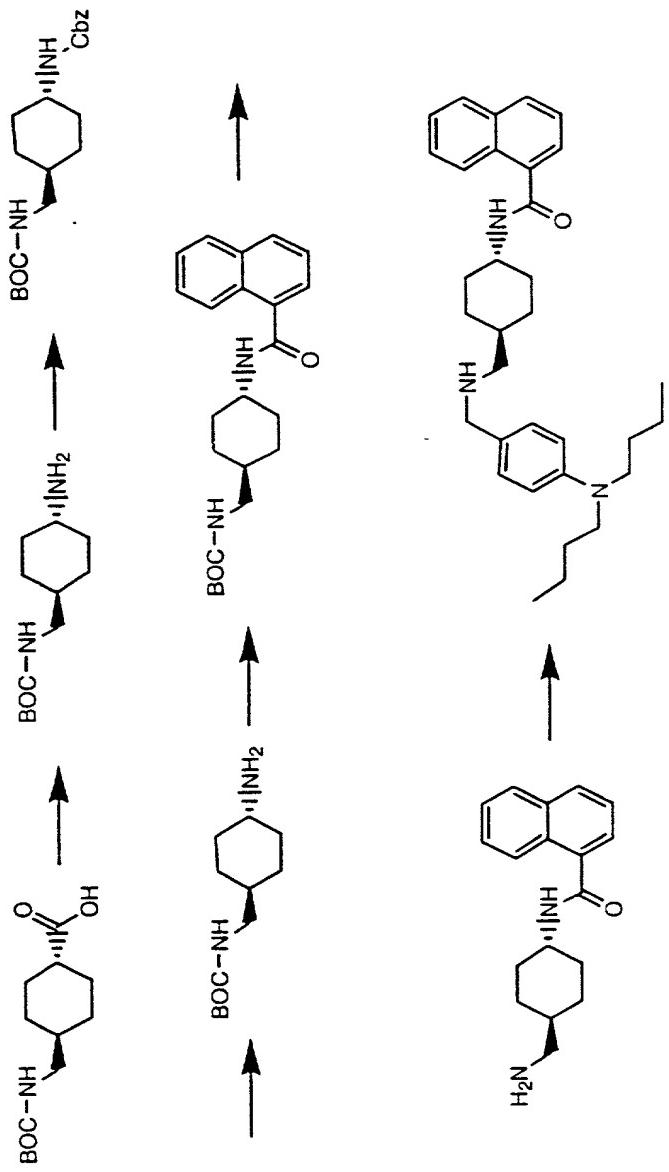
iii. DBU

iv. p-nitrophenylchloroformate

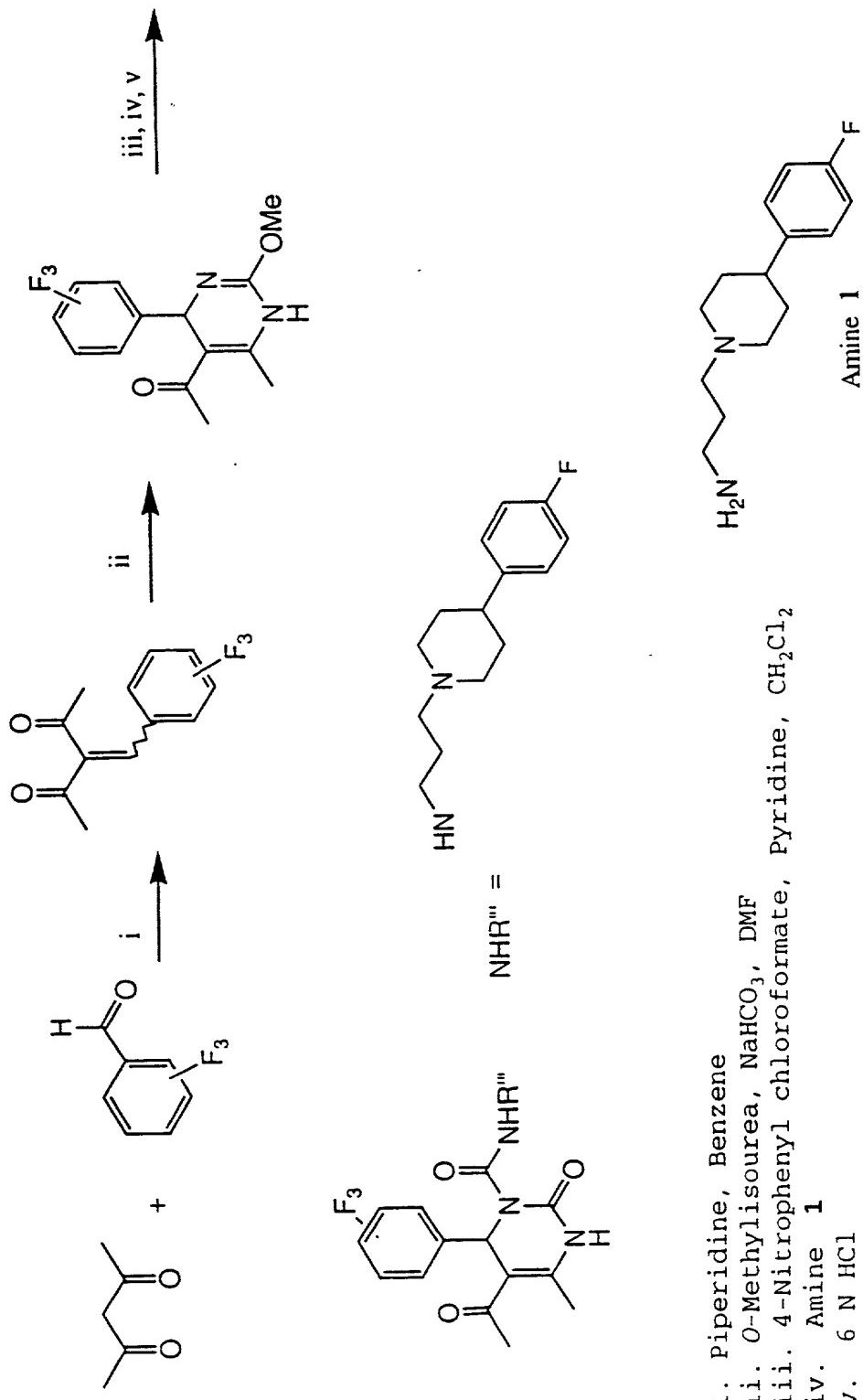
Scheme 7. Synthesis of Example 5 and Analogs



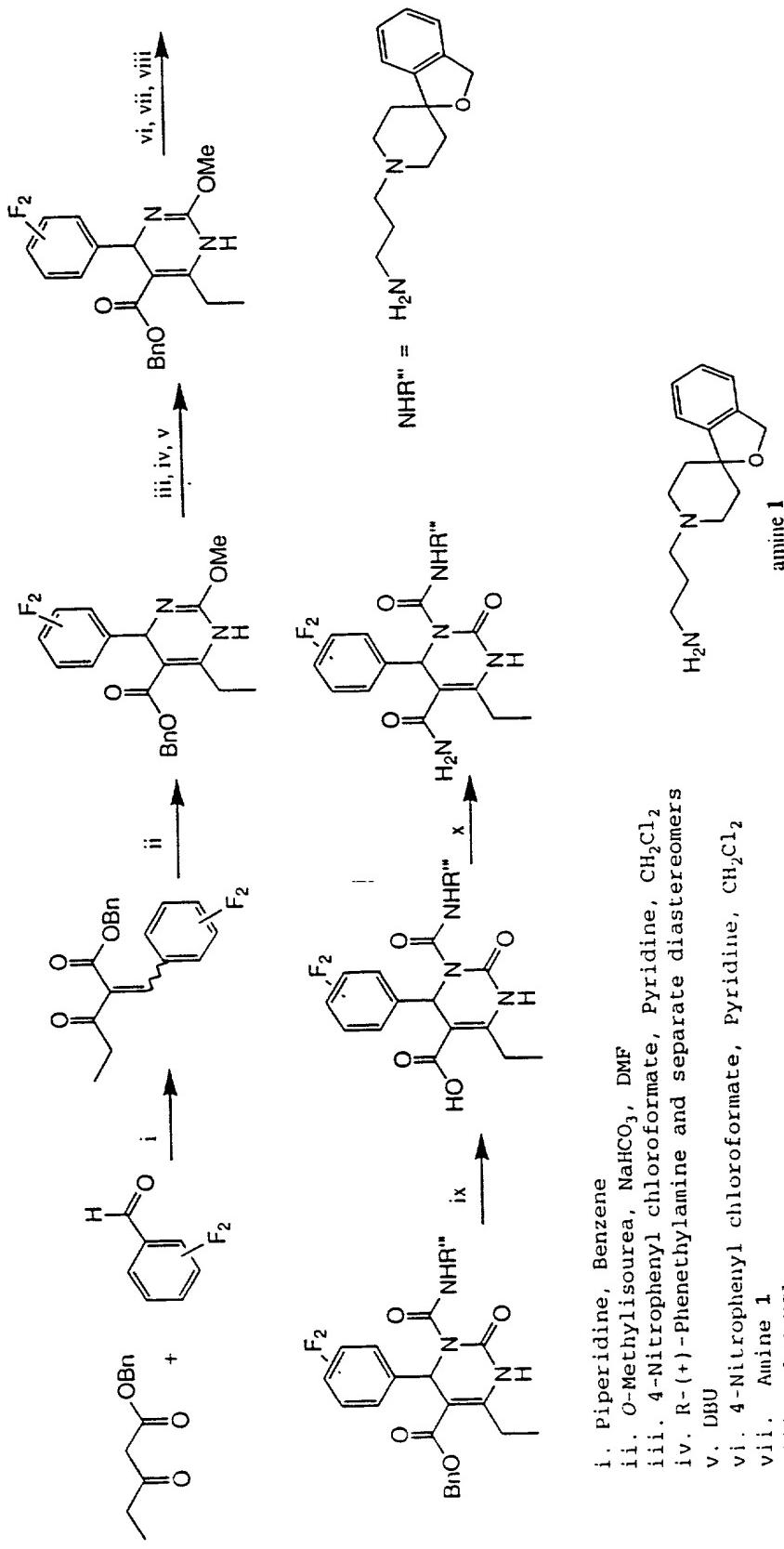
Scheme 8. Synthesis of Example 13



Scheme 9. Synthesis of Example 12

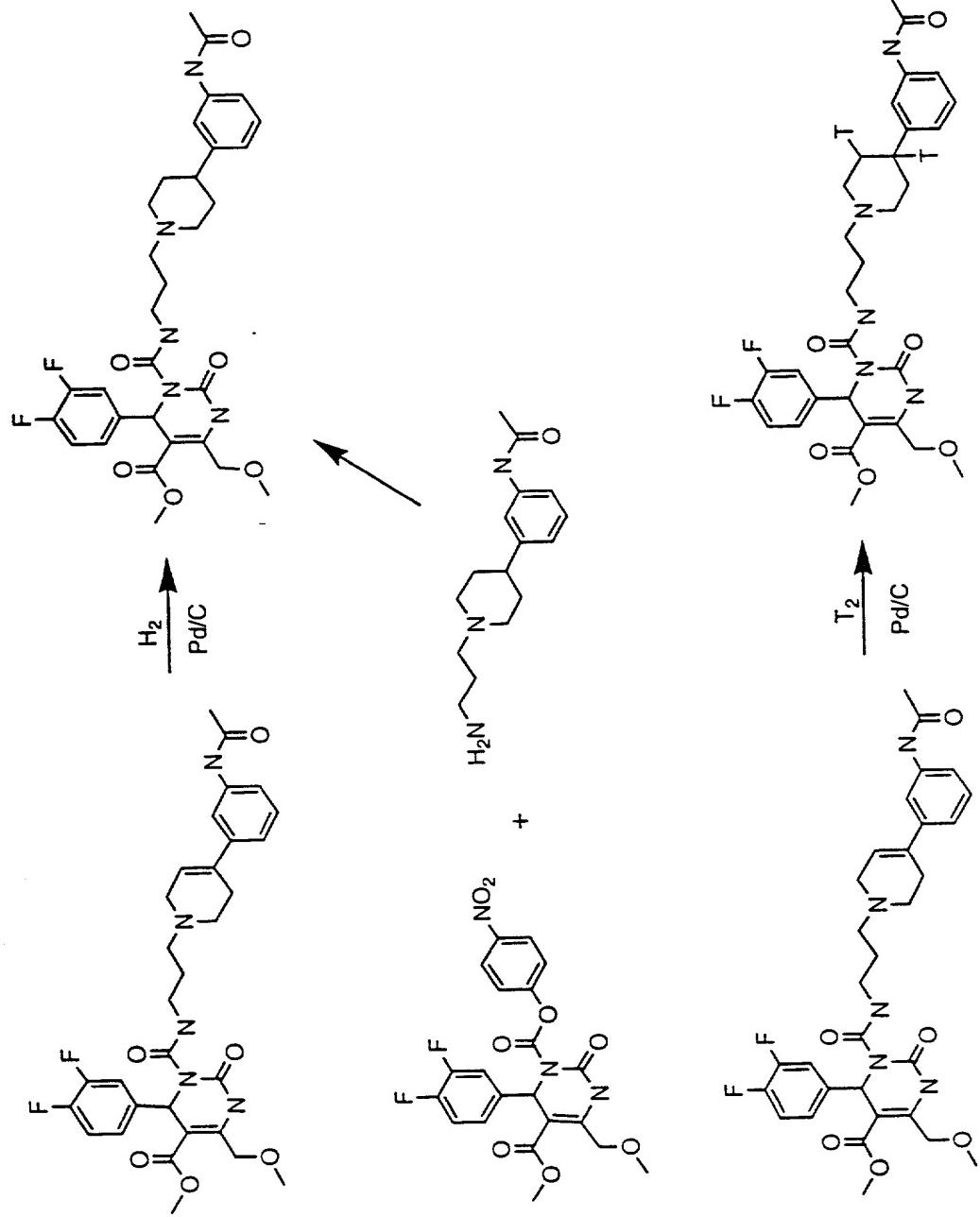


Scheme 10. Synthesis of Examples 4 and 22.

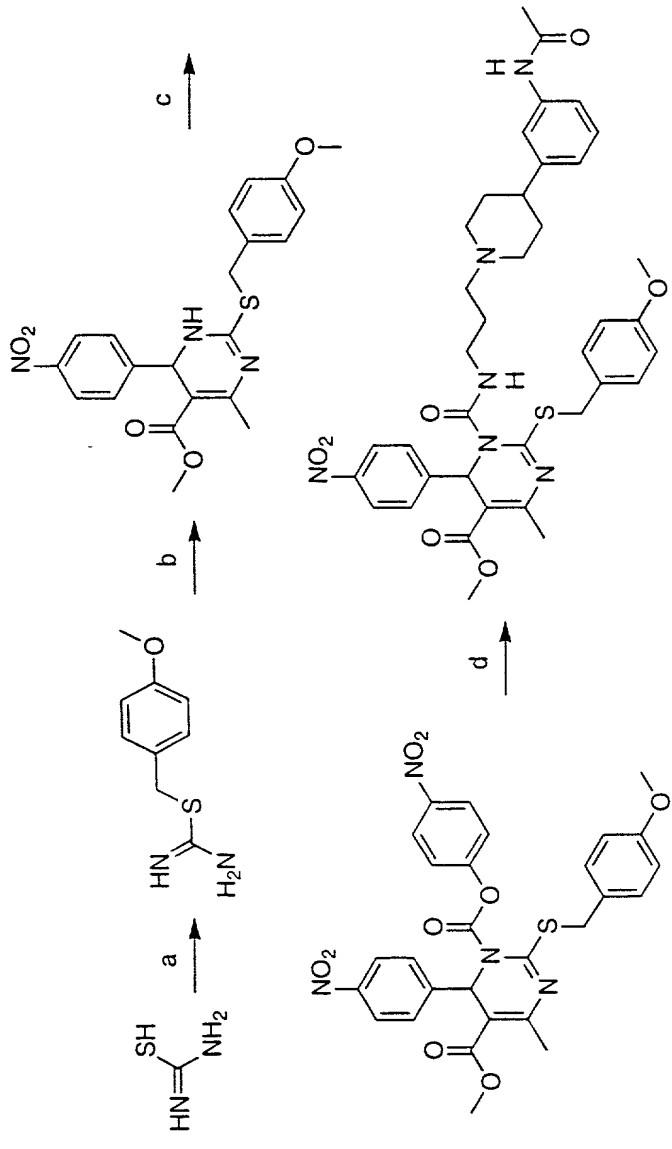


- i. Piperidine, Benzene
- ii. O-Methylisourea, NaHCO_3 , DMF
- iii. 4-Nitrophenyl chloroformate, Pyridine, CH_2Cl_2
- iv. R-(+)-Phenethylamine and separate diastereomers
- v. DBU
- vi. 4-Nitrophenyl chloroformate, Pyridine, CH_2Cl_2
- vii. Amine 1
- viii. 6 N HCl
- ix. H_2 , Pd-C, MeOH/water
- x. EDC, NMM, NH_4OH , CH_2Cl_2

Scheme 11. Synthesis of Example 10 and its Tritiated Analog

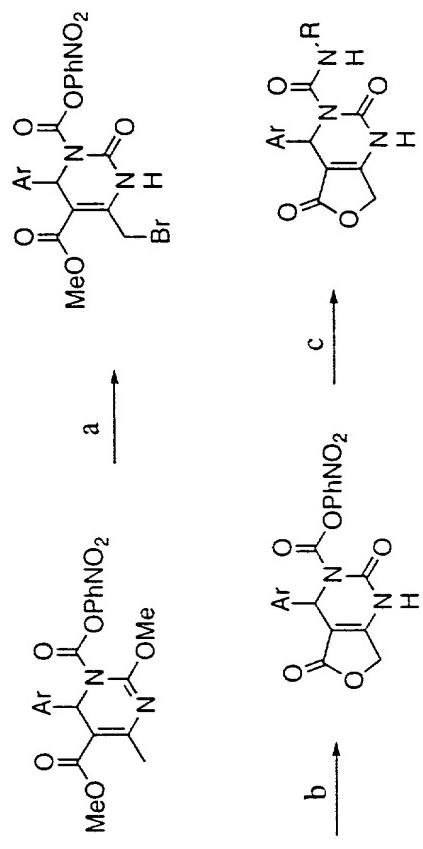


Scheme 12: Synthesis of Dihydropyrimidines

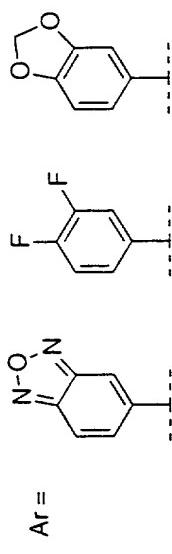


- a. p-methoxybenzyl chloride, THF, 0 to 65 °C;
- b. Methyl 2-{(4-nitrophenyl)methylene}-3-oxobutyrate (prepared from p-nitrobenzaldehyde, methyl acetoacetate, piperidinium acetate in isopropanol), NaOAc, DMF, 65 °C;
- c. p-nitrophenyl chloroformate, NaHCO₃, dichloromethane
- d. N-(3-{1-(3-aminopropyl)-4-piperidinyl}phenyl)acetamide

Scheme 13. Synthesis of Dihydropyrimidinone Fused Lactones



(a) $\text{Br}_2, \text{CHCl}_3$ (b) Heat, 130 $^{\circ}\text{C}$ (c) RNH_2 , THF or CH_2Cl_2 , 60–80% yield overall.



Scheme 14: Synthesis of Substituted Dihydropyrimidinones and Reverse Dihydropyrimidinones

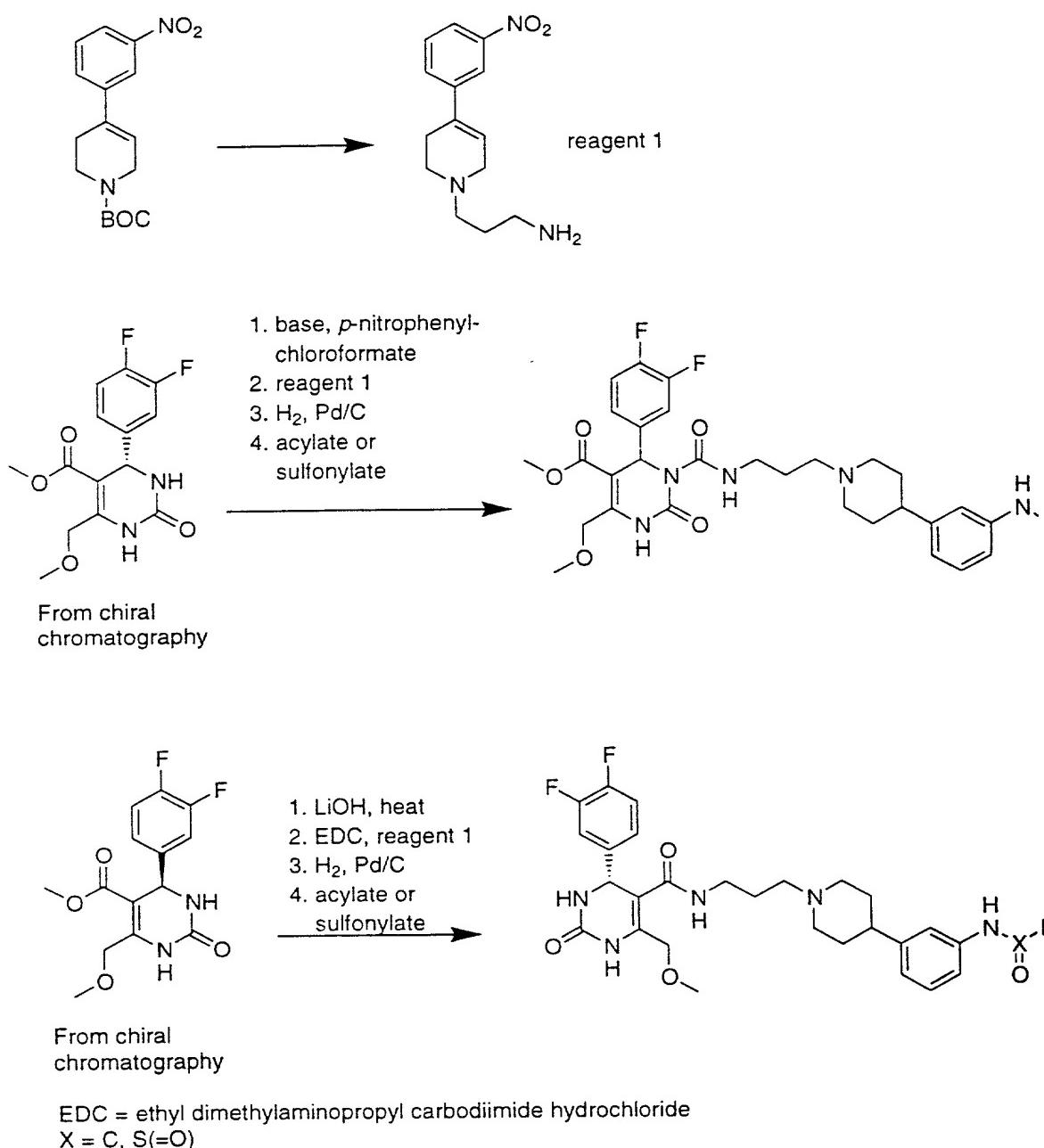
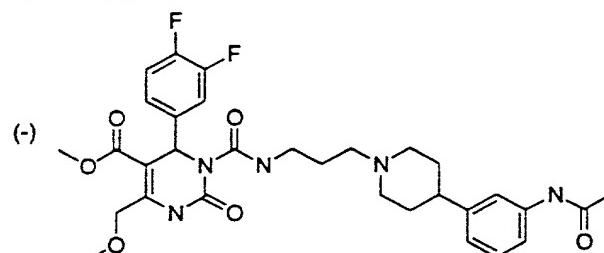
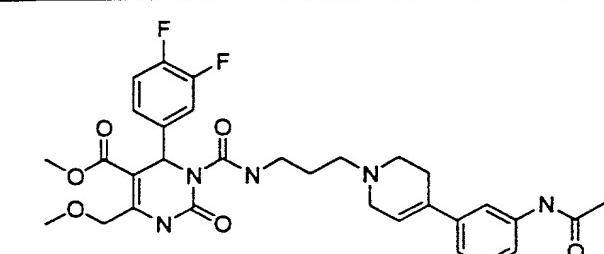
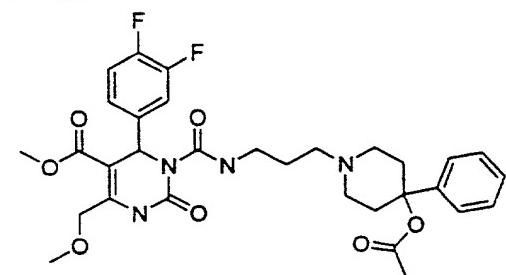
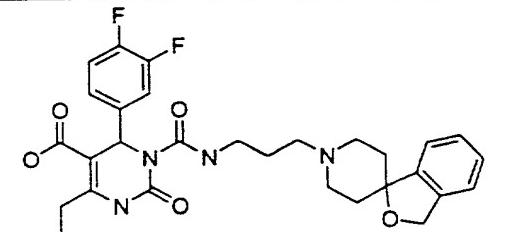
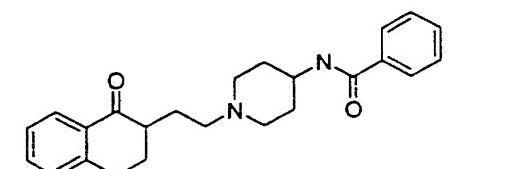
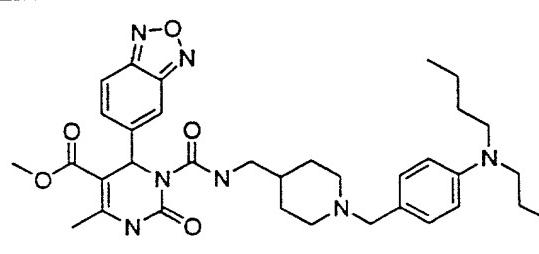
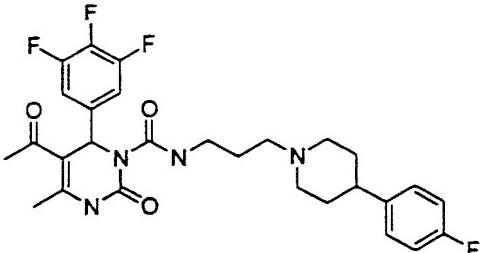
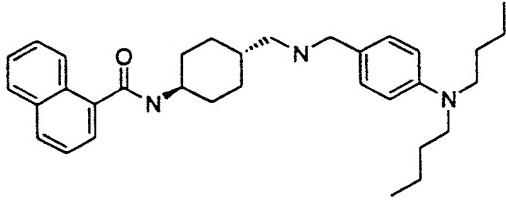
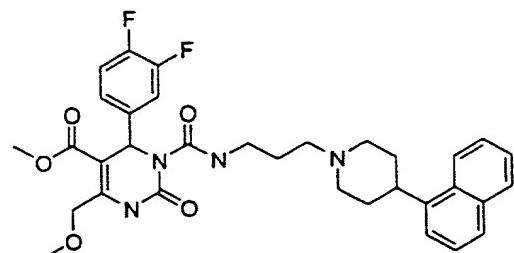
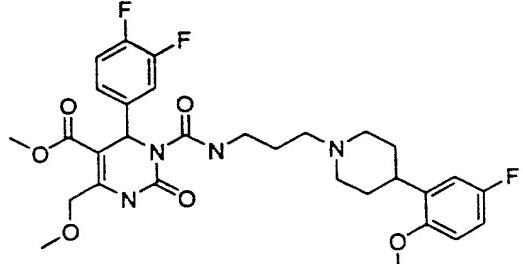
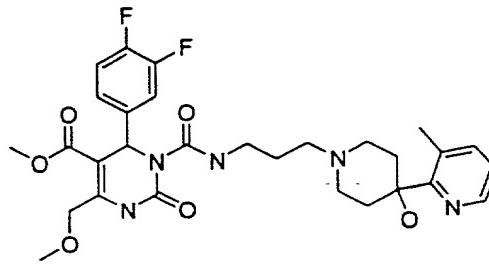
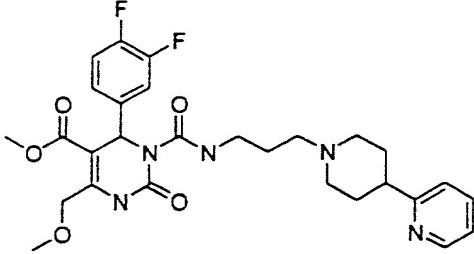
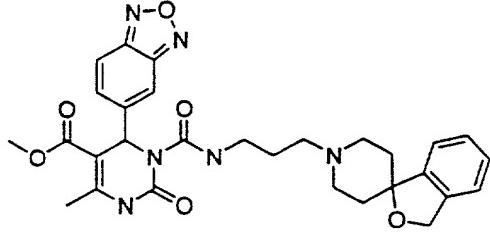
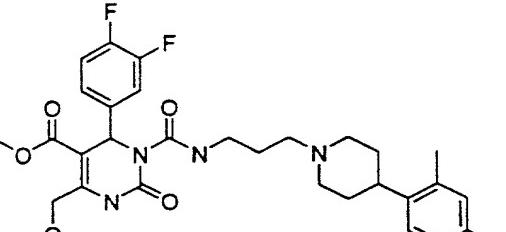
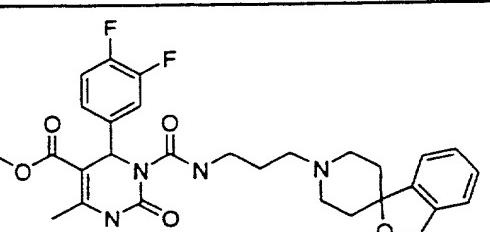
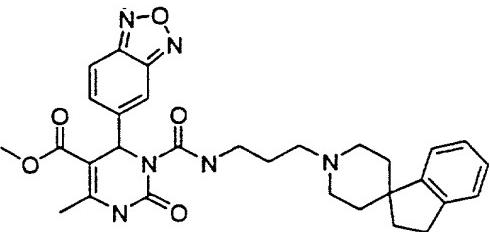
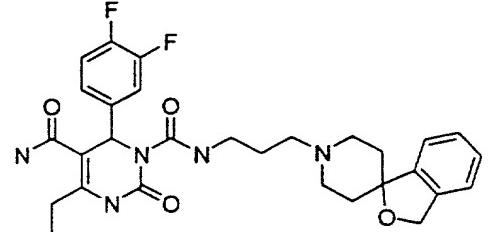


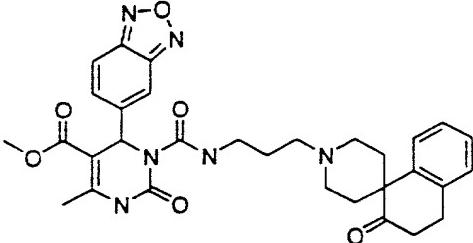
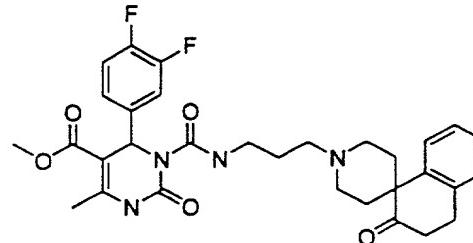
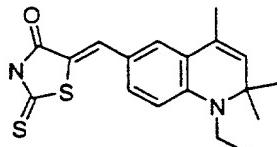
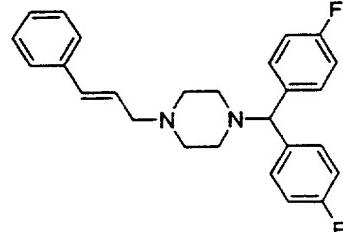
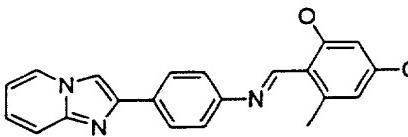
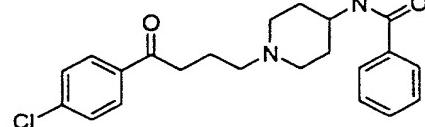
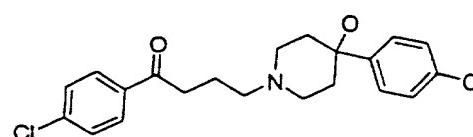
Table 1

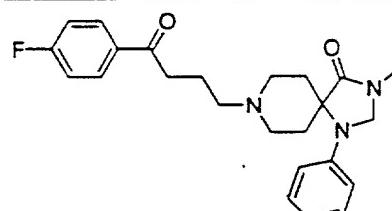
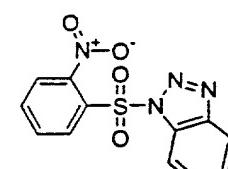
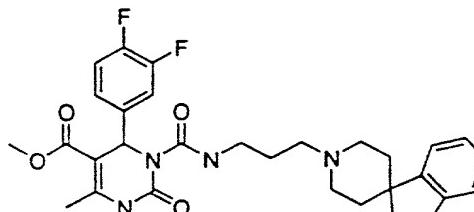
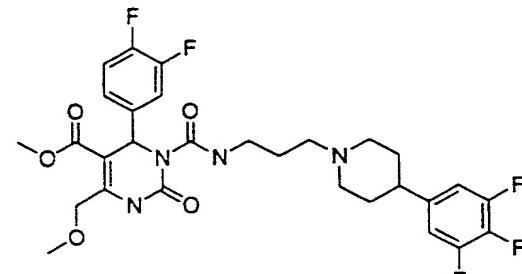
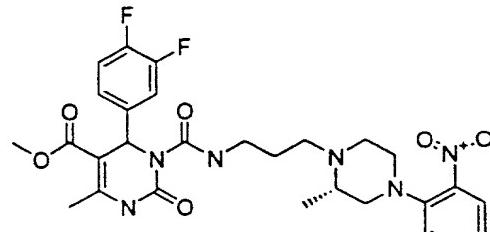
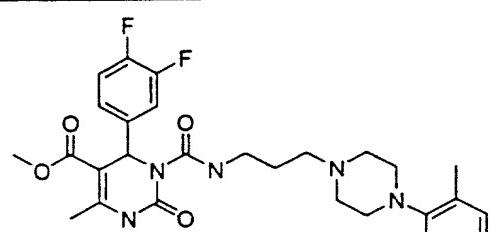
EXAMPLE NO.	STRUCTURE	K _B (nM) hMCH1
1		42
2		18
3		201
4		187
5		258
6		42

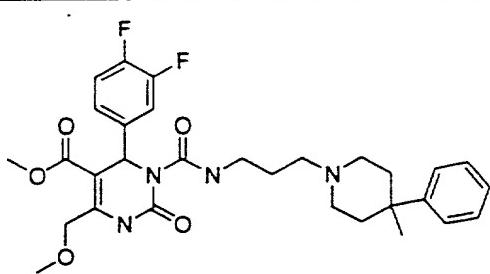
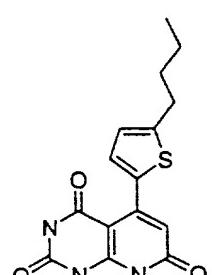
EXAMPLE No.	STRUCTURE	K _b (nM) hMCH1
7		41
8		88
9		35
10	<p>(+)</p>	0.3
11		331

EXAMPLE NO.	STRUCTURE	K _b (nM) hMCH1
12		29
13		284
14		2
15		289
16		329

EXAMPLE NO.	STRUCTURE	K _B (nM) hMCH1
17		373
18		1
19		7
20		5
21		28
22		40

EXAMPLE NO.	STRUCTURE	K _b (nM) hMCH1
23		68
24		102
25		126
26		260
27		279
28		60
29		9

EXAMPLE NO.	STRUCTURE	K _b (nM) hMCH1
30		479
31		7
32		67
33		12
34		182
35		276

EXAMPLE NO.	STRUCTURE	K _b (nM) hMCH1
36		406
37		162

General Methods. : All reactions (except for those done by parallel synthesis reaction arrays) were performed under an Argon atmosphere and the reagents, neat or in appropriate solvents, were transferred to the reaction vessel via syringe and cannula techniques. The parallel synthesis reaction arrays were performed in vials (without an inert atmosphere) using J-KEM heating shakers (Saint Louis, MO). Anhydrous solvents were purchased from Aldrich Chemical Company and used as received. The examples described in the patent were named using ACD/Name program (version 2.51, Advanced Chemistry Development Inc., Toronto, Ontario, M5H2L3, Canada). Unless otherwise noted, the ^1H spectra were recorded at 300 and 400 MHz (QE Plus and Brüker respectively) with tetramethylsilane as internal standard. s = singlet; d = doublet; t = triplet; q = quartet; p = pentet; sext; sept; br = broad; m = multiplet. Elemental analyses were performed by Robertson Microlit Laboratories, Inc. Unless otherwise noted, mass spectra were obtained using low-resolution electrospray (ESMS) and MH^+ is reported. Thin-layer chromatography (TLC) was carried out on glass plates precoated with silica gel 60 F₂₅₄ (0.25 mm, EM Separations Tech.). Preparative thin-layer chromatography was carried out on glass sheets precoated with silica gel GF (2 mm, Analtech). Flash column chromatography was performed on Merck silica gel 60 (230 - 400 mesh). Melting points (mp) were determined in open capillary tubes on a Mel-Temp apparatus and are uncorrected.

Piperidine Side Chain Intermediates

TERT-BUTYL 4-{[(TRIFLUOROMETHYL) SULFONYL]OXY}-1,2,3,6-TETRAHYDRO-1-PYRIDINECARBOXYLATE:

n-Butyl lithium (17.6 mL, 44.2 mmol, 2.5 M in hexanes) was added to a solution of diisopropyl amine (96.2 mL, 44.2 mmol) in 40 mL of dry THF at 0 °C and stirred for 20 minutes. The reaction mixture was cooled to -78 °C and tert-butyl 4-oxo-1-piperidinecarboxylate (Aldrich Chemical Company, 40.0 mmol) in THF (40 mL) was added dropwise to the reaction mixture and stirred for 30 minutes. Tf₂NPh (42.0 mmol, 15.0 g) in THF (40 mL) was added dropwise to the reaction mixture and stirred at °C overnight. The reaction mixture was concentrated *in vacuo*, re-dissolved in hexanes:EtOAc (9:1), passed through a plug of alumina and the alumina plug was washed with hexanes:EtOAc (9:1). The combined extracts were concentrated to yield 16.5 g of the desired product that was contaminated with some starting Tf₂NPh.

¹H NMR (400 MHz, 400 MHz, CDCl₃) δ 5.77 (s, 1 H), 4.05 (dm, 2 H, J=3.0 Hz), 3.63 (t, 2 H, J=5.7 Hz), 2.45 (m, 2 H), 1.47 (s, 9 H).

TERT-BUTYL 4-[3-(AMINO) PHENYL]-1,2,3,6-TETRAHYDRO-1-PYRIDINECARBOXYLATE:

A mixture of 2 M aqueous Na₂CO₃ solution (4.2 mL), tert-butyl 4-{[(trifluoromethyl)sulfonyl]oxy}-1,2,3,6-tetrahydro-1-pyridine-carboxylate (0.500 g, 1.51 mmol), 3-aminophenylboronic acid hemisulfate (0.393 g, 2.11 mmol), lithium chloride (0.191 g, 4.50 mmol) and tetrakis-triphenylphosphine palladium (0) (0.080 g, 0.075 mmol) in dimethoxyethane (5 mL) was heated at reflux temperature for 3 hours, under an inert atmosphere (an initial degassing of the mixture is recommended to prevent the formation of

triphenylphosphine oxide). The organic layer of the cooled reaction mixture was separated and the aqueous layer was washed with ethyl acetate (3X). The combined organic extracts were dried and concentrated *in vacuo*.
5 The crude product was chromatographed (silica, hexanes:EtOAc:dichloromethane (6:1:1) with 1% added isopropylamine to protect the BOC group from hydrolysis) to give 0.330 g of the desired product in 81% yield:
10 ^1H NMR (400 MHz, CDCl_3) δ 7.12 (t, 1H, $J = 7.60$ Hz), 6.78 (d, 1H, $J = 8.4$ Hz), 6.69 (t, 1H, $J = 2.0$ Hz), 6.59 (dd, 1H, $J = 2.2, 8.0$ Hz), 6.01 (m, 1H), 4.10 - 4.01 (d, 2H, $J = 2.4$ Hz), 3.61 (t, 2H, $J = 5.6$ Hz), 2.52 - 2.46 (m, 2H), 1.49 (s, 9H); ESMS m/e : 275.2 ($M + H$)⁺.
15 Anal. Calc. for $\text{C}_{16}\text{H}_{24}\text{N}_2\text{O}_2$: C, 70.04; H, 8.08; N, 10.21.
Found: C, 69.78; H, 7.80; N, 9.92.

TERT-BUTYL 4-[3-(AMINO)PHENYL]-1-PIPERIDINECARBOXYLATE
A mixture of 3.10 g of tert-butyl 4-(3-aminophenyl)-
1,2,3,6-tetrahydropyridine-1-carboxylate (11.3 mmol) and
20 1.0 g of 10% Pd/C in 200 mL of ethanol was hydrogenated
at room temperature using the balloon method for 2 days.
The reaction mixture was filtered and washed with
ethanol. The combined ethanol extracts were
concentrated *in vacuo* and the residue was
25 chromatographed on silica (dichloromethane: methanol
95:5 with 1% isopropylamine added to protect the BOC
group from hydrolysis) to give 2.63 g of the desired
product (84%).

30 **TERT-BUTYL 4-[3-(ACETYLAMINO)PHENYL]-1,2,3,6-TETRAHYDRO-
1-PYRIDINECARBOXYLATE:** A mixture of saturated of
aqueous Na_2CO_3 solution (25 mL), tert-butyl 4-
{[(trifluoromethyl)sulfonyl]oxy}-1,2,3,6-tetrahydro-1-

pyridine-carboxylate (20 mmol), 3-acetamidophenylboronic acid (30 mmol) and tetrakis-triphenylphosphine palladium (0) (1.15 g) and dimethoxyethane (40 mL) was heated at reflux temperature overnight. The organic layer of the cooled reaction mixture was separated and the aqueous layer was washed with ethyl acetate (3X). The combined organic extracts were dried and concentrated *in vacuo*. The crude product was chromatographed, giving the desired product: ^1H NMR (CDCl_3) δ 8.11 (br s, 1 H), 7.57 (br s, 1 H), 7.41 (br d, 1 H, $J=7.8$ Hz), 7.25 (apparent t, 1 H, $J=7.8$ Hz), 7.08 (br d, 1 H, $J=7.8$ Hz), 5.99 (br s, 1 H), 4.03 (br m, 2 H, $J=2.7$ Hz), 3.59 (t, 2 H, $J=5.7$ Hz), 2.46 (m, 2 H), 2.16 (s, 3 H), 1.49 (s, 9 H).

15 **N1-[3-(1,2,3,6-TETRAHYDRO-4-PYRIDINYL)PHENYL]ACETAMIDE:**

A solution of 4 M HCl in dioxane (10 mL) was added to tert-butyl 4-[3-(acetylamino)phenyl]-1,2,3,6-tetrahydro-1-pyridinecarboxylate (8.25 mmol) in dichloromethane (30 mL). The reaction mixture was stirred at room temperature overnight, concentrated *in vacuo*, giving the desired product as the hydrochloride salt (2.1 g): ^1H NMR (CDCl_3) δ 7.41-7.00 (m, 4 H), 6.10 (br, 1 H), 3.55 (m, 2 H), 3.16 (t, 2 H, $J = 5.7$ Hz), 2.44 (m, 2 H), 2.19 (s, 3 H).

25

TERT-BUTYL N-(3-BROMOPROPYL)CARBAMATE: Prepared from 3-bromopropylamine hydrobromide and BOC_2O in the presence of base in dichloromethane, 9.89 mmol: ^1H NMR (CDCl_3) δ 5.07 (br, 1 H), 3.31 (t, 2 H, $J=6.6$ Hz), 3.12 (apparent br q, 2 H, $J=6.0$ Hz), 1.92 (p, 2 H, $J=6.6$ Hz), 1.30 (s, 9H).

TERT-BUTYL N-(3-[4-[3-(ACETYLAMINO)PHENYL]-1,2,3,6-TETRAHYDRO-1-PYRIDINYL]PROPYL)CARBAMATE: A solution of N1-[3-(1,2,3,6-tetrahydro-4-pyridinyl)phenyl]acetamide.HCl (8.24 mmol), tert-butyl 5 N-(3-bromopropyl)carbamate and potassium carbonate (33 mmol) in dry dioxane (30 mL) was heated at reflux temperature overnight. The solids were removed by filtration, the solution was concentrated *in vacuo* and the product was chromatographed, giving the desired 10 product (110 mg).

TERT-BUTYL N-(3-4-[3-(ACETYLAMINO)PHENYL]-1,2,3,6-TETRAHYDRO-1-PYRIDINYLPROPYL)CARBAMATE: ^1H NMR (CDCl_3) δ 7.65 (s, 1 H), 6.98 (s, 1 H), 7.45 (d, 1 H, $J=7.8$ Hz), 15 7.16 (apparent t, 1 H, $J=7.8$ Hz), 7.10 (d, 1 H, $J=7.8$ Hz), 6.02 (s, 1 H), 5.23 (b, 1 H), 3.40 (b, 2 H), 3.30-1.80 (m, 10 H), 2.18 (s, 3 H), 1.45 (s, 9 H).

N1-[3-[1-(3-AMINOPROPYL)-1,2,3,6-TETRAHYDRO-4-PYRIDINYL]PHENYL]ACETAMIDE: A 1:1 solution of TFA: CH_2Cl_2 (5 mL) was added to tert-butyl N-(3-[4-[3-(acetylamino)phenyl]-1,2,3,6-tetrahydro-1-pyridinyl]propyl)carbamate in dichloromethane (5 mL). The resulting solution was stirred at room temperature for 1-3 days, saturated NaHCO_3 was added until pH > 6, the organic layer was separated, and dried *in vacuo*, giving the desired product (45 mg):

N1-[3-[1-(3-AMINOPROPYL)-1,2,3,6-TETRAHYDRO-4-PYRIDINYL]PHENYL]ACETAMIDE: From N1-[3-[1-(3-aminopropyl)-1,2,3,6-tetrahydro-4-pyridinyl]phenyl]acetamide and acid (TFA or HCl), followed by basification of the resulting salt: ^1H NMR

(CDCl₃) δ 7.68 (br, 1 H), 7.35 (dm, 1 H, J=7.8 Hz), 7.25 (apparent t, 1 H, J=7.8 Hz), 7.15 (dm, 1 H, J=7.8 Hz), 6.12 (m, 1 H), 3.22 (m, 2 H), 3.03 (t, 2 H, J=7.3 Hz), 2.78 (t, 2 H, J=5.5 Hz), 2.70-2.50 (m, 4 H), 2.10 (s, 3 H), 1.87 (p, 2 H, J=7.3 Hz).

TERT-BUTYL 4-[3-(ACETYLAMINO)PHENYL]-1-PIPERIDINECARBOXYLATE: A mixture *tert*-butyl 4-[3-(acetylamino)phenyl]-1,2,3,6-tetrahydro-1-pyridinecarboxylate (710 mg) and 5% Pd/C (100 mg) in EtOH (10 mL) was hydrogenated (balloon technique) at room temperature overnight. The reaction mixture was passed through a pad of Celite 545 and the pad of Celite was washed with ethanol. The combined ethanol extracts were concentrated and chromatographed, giving the desired product (660 mg): ¹H NMR (CDCl₃) δ 7.80 (s, 1 H), 7.41-7.20 (m, 3 H), 6.94 (d, 1 H, J=7.5 Hz), 4.21 (m, 2 H), 2.75 (m, 2 H), 2.62 (m, 1 H), 2.16 (s, 3 H), 1.78 (m, 2 H), 1.56 (m, 2 H), 1.48 (s, 9 H).

N1-[3-(4-PIPERIDYL)PHENYL]ACETAMIDE: A solution of HCl in dioxane (4N, 5 mL) was added to *tert*-butyl 4-[3-(acetylamino)phenyl]-1-piperidinecarboxylate (660 mg) in dry dichloromethane (15 mL). The reaction mixture was stirred at room temperature overnight and concentrated *in vacuo*, giving the desired product (550 mg): mp 102-104 °C; ¹H NMR (CDCl₃) δ 2.02 (d, J=13.2 Hz, 2H), 2.11-2.45 (m, 5H), 2.67-2.77 (m, 1H), 3.00-3.10 (m, 2H), 3.51 (d, J=10.5 Hz, 2H), 6.94 (d, J=7.5 Hz, 1H), 7.20-7.46 (m, 3H), 7.60 (s, 1H); Anal. Calcd. For C₁₃H₁₉N₂OCl+0.86 CH₂Cl₂: C, 50.78; H, 6.37; N, 8.55. Found: C, 50.80; H, 7.55; N, 7.01.

TERT-BUTYL

N- (3- {4- [3-

(ACETYLAMINO) PHENYL] PIPERIDINO} PROPYL) CARBAMATE:

A

solution of *N*1-[3-(4-piperidyl)phenyl]acetamide (550 mg, 0.210 mmol), *tert*-butyl *N*-(3-bromopropyl)carbamate (550 mg, 0.230 mmol), K_2CO_3 (1.10 g, 0.890 mmol), diisopropylethyl amine (1.50 mL) and a few crystals of KI in dioxane (20 mL) was heated at reflux temperature for 2 days. The precipitated salts were removed by filtration, concentrated *in vacuo* and the crude product was chromatographed, giving the desired product (340 mg): 1H NMR ($CDCl_3$) δ 8.15 (s, 1 H), 7.47-7.44 (m, 2 H), 7.22 (t, 1 H, $J=7.8$ Hz), 6.94 (d, 1 H, $J=7.8$ Hz), 5.53 (b, 1 H), 3.23 (b, 6 H), 2.80-1.60 (m, 9 H), 2.20 (s, 3 H), 1.45 (s, 9 H).

15

***N*1-{3- [1- (3-AMINOPROPYL) -4-PIPERIDYL] PHENYL}ACETAMIDE:**

TFA (1.0 mL) was added to a solution of *tert*-butyl *N*-(3-{4-[3-(acetylamino)phenyl]piperidino}propyl)carbamate (340 mg) in dry dichloromethane (10 mL) and stirred at room temperature for 5 h. A 10% aqueous solution of KOH was added to the reaction mixture until pH > 6 and then the dichloromethane was removed *in vacuo*. The aqueous layer was frozen and lyophilized to give a solid, which was extracted with methanol. Removal of the solvent gave the desired product (120 mg) as an oil: 1H NMR ($CDCl_3$) δ 7.23-7.16 (apparent t, 1H, $J=7.5$ Hz), 6.95-6.92 (m, 1H), 3.03-2.99 (m, 2H), 2.77-2.73 (t, 2H, $J = 6.6$ Hz), 2.50-1.60 (m, 10 H), 2.13 (s, 3 H).

30

TERT-BUTYL 4- (3-NITROPHENYL) -3,6-DIHYDRO-1(2H) - PYRIDINECARBOXYLATE

1H NMR (400 MHz, 400 MHz, $CDCl_3$) δ 8.23 (s, 1H), 8.11 (d, 1H, $J=8.0$ Hz), 7.69 (d, 1H, $J=8.0$ Hz), 7.51 (t, 1H,

J=8.0 Hz), 6.20 (m, 1H), 4.17-4.08 (m, 2H), 3.67 (t, 2H, J=5.6 Hz), 2.61-2.52 (m, 2H), 1.50 (s, 9H); ESMS m/e : 249.1 (M + H - C₄H₈)⁺.

5 **1,2,3,6-TETRAHYDRO-4-(3-NITROPHENYL) PYRIDINE:** Into a stirred solution of 5.00 g (16.0 mmol) of *tert*-butyl 1,2,3,6-tetrahydro-4-(3-nitrophenyl)pyridine-1-carboxylate in 100 ml of 1,4-dioxane at 0°C was bubbled HCl gas for 10 minutes. The reaction mixture was
10 allowed to warm to room temperature and the bubbling of the HCl gas was continued for an additional 1 hour. The solvent was removed *in vacuo*, the residue was dissolved in 50 mL of water and was neutralized by the addition of KOH pellets. The aqueous solution was extracted with 3 X 80 mL of dichloromethane and the combined organic extracts were dried (MgSO₄), filtered and concentrated *in vacuo*. The residue was purified by column chromatography (silica, 9 : 1 ,dichloromethane :
15 methanol + 1% isopropyl amine) to afford 2.85 g (87.5% yield) of the desired product: ¹H NMR (400 MHz, 400 MHz, CDCl₃) δ 8.24 (s, 1H), 8.09 (d, 1H, J=8.4 Hz), 7.71 (d, 1H, J=8.0 Hz), 7.49 (t, 1H, J=8.0 Hz), 6.35-6.25 (m, 1H), 3.58 (apparent q, 2H, J=3.0 Hz), 3.14 (t, 2H, J=5.6 Hz), 2.54-2.46 (m, 2H).

25 **TERT-BUTYL 3-(4-(3-NITROPHENYL)-3,6-DIHYDRO-1(2H)-PYRIDINYL) PROPYLCARBAMATE:** A mixture of 2.80 g (14.0 mmol) of 1,2,3,6-tetrahydro-4-(3-nitrophenyl)pyridine, 3.60 g (15.0 mmol) of *tert*-butyl N-(3-bromopropyl)carbamate, 11.6 g (84.0 mmol) of K₂CO₃, 14.6 mL (84.0 mmol) of diisopropylethylamine and 0.78 g (2.00 mmol) of tetrabutylammonium iodide in 250 mL of 1,4-dioxane was heated at reflux temperature for 14 hours.

The reaction mixture was filtered and the filtrate was dried ($MgSO_4$), concentrated *in vacuo* and the residue was purified by column chromatography (silica, 9:1, dichloromethane: methanol + 1% isopropyl amine) to afford 4.35 g (85.7% yield) of the desired product: 1H NMR (400 MHz, 400 MHz, $CDCl_3$) δ 8.24 (t, 1H, $J=1.9$ Hz), 8.09 (dd, 1H, $J=1.9$, 8.0 Hz), 7.70 (apparent d, 1H, $J=8.0$ Hz), 7.49 (t, 1H, $J=8.0$ Hz), 6.23 (m, 1H), 3.29-3.18 (m, 4H), 2.75 (t, 2H, $J=5.6$ Hz), 2.64-2.54 (m, 4H), 1.82-1.70 (m, 2H), 1.44 (s, 9H); ESMS m/e : 362.2 (M + H) $^+$.

3 - (4 - (3 - NITROPHENYL) - 3,6 - DIHYDRO - 1 (2H) - PYRIDINYL) - 1 - PROPANAMINE: Into a stirred solution of 4.35 (12.0 mmol) of tert-butyl 3-(4-(3-nitrophenyl)-3,6-dihydro-1(2H)-pyridinyl)propylcarbamate in 100 ml of 1,4-dioxane at 0°C was bubbled HCl gas for 10 minutes. The reaction mixture was allowed to warm to room temperature and the bubbling was continued for an additional 1 hour. The solvent was removed *in vacuo*, the residue was dissolved in 50 mL of water and was neutralized by the addition of KOH pellets. The aqueous solution was extracted with 3 X 80 mL of dichloromethane, the combined organic extracts were dried ($MgSO_4$), filtered and concentrated *in vacuo*. The residue was purified by column chromatography (silica, 9 : 1 ,dichloromethane : methanol + 1% isopropyl amine) to afford 3.05 g (97.0% yield) of the desired product: 1H NMR (400 MHz, 400 MHz, $CDCl_3$) δ 8.24 (t, 1H, $J=1.8$ Hz), 8.09 (dd, 1H, $J=1.8$, 8.2 Hz), 7.69 (dd, 1H, $J=1.8$, 8.2 Hz), 7.48 (t, 1H, $J=8.2$ Hz), 6.24 (m, 1H), 3.21 (d, 2H, $J=3.6$ Hz), 2.84 (t, 2H, $J=6.6$ Hz), 2.75 (t, 2H, $J=5.8$ Hz), 2.64-2.54 (m, 4H), 1.76 (m, 2H); ESMS m/e : 262.2 (M + H) $^+$; Anal. Calc.

for C₁₄H₁₉N₃O₂ (0.06 CHCl₃): C, 62.90; H, 7.16; N, 15.65.
Found: C, 63.20; H, 7.16; N, 15.65.

METHYL (4S)-3-[({3-[4-(3-AMINOPHENYL)-1-
5 PIPERIDINYLPROPYL]AMINO)CARBOXYL]-4-(3,4-
DIFLUOROPHENYL)-6-(METHOXYMETHYL)-2-OXO-1,2,3,4-
TETRAHYDRO-5-PYRIMIDINECARBOXYLATE: A mixture of 3.02 g
(6.33 mmol) 5-methyl 1-(4-nitrophenyl) (6S)-6-(3,4-
difluorophenyl)-4-(methoxymethyl)-2-oxo-3,6-dihydro-
10 1,5(2H)-pyrimidinedicarboxylate, 1.50 g (5.80 mmol) of
3-(4-(3-nitrophenyl)-3,6-dihydro-1(2H)-pyridinyl)-1-
propanamine, 7.94 g (75.5 mmol) of K₂CO₃ and 1.00 mL of
methanol in 200 mL dichloromethane (under argon) was
stirred at room temperature for 1 hour. The reaction
mixture was filtered and concentrated *in vacuo*. The residue
15 was dissolved in 100 mL of ethyl acetate and washed 3 X 50 mL of 5% aqueous NaOH solution, the organic layer was dried (MgSO₄) and concentrated *in vacuo*. The residue was dissolved in 100 mL of anhydrous ethanol containing 0.50 g 10% Pd/C and the reaction mixture was stirred under a hydrogen balloon for 24 hours. The reaction mixture was passed through a column of Celite 545 filtering agent, washed with ethanol, the filtrate was dried (MgSO₄) and concentrated *in vacuo*.
20 The residue was purified by column chromatography (silica, 9.5 : 0.5 , dichloromethane : methanol + 1% isopropyl amine) to afford 1.65 g (52.0% yield) of the desired product.

30 TERT-BUTYL 4-[3-(ISOBUTYRYLAMINO)PHENYL]-3,6-DIHYDRO-
1(2H)-PYRIDINECARBOXYLATE: Into a solution of 4.00 g (16.0 mmol) of tert-butyl 4-(3-aminophenyl)-3,6-dihydro-1(2H)-pyridinecarboxylate and 5.60 mL (32.0 mmol) of

diisopropylethylamine in 100 mL dichloromethane was slowly added 1.90 mL (19.0 mmol) of isobutyryl chloride. The reaction mixture was stirred at room temperature for 2 hours, washed with water, dried ($MgSO_4$), and concentrated *in vacuo*. The residue was purified by column chromatography (silica, 50 : 46 : 3 : 1, hexanes : dichloromethane : methanol : isopropyl amine) to afford 2.90 g (52.0% yield) of the desired product: 1H NMR (400 MHz, $CDCl_3$) δ 7.69 (s, 1 H), 7.34 (d, 1 H, $J=7.8$ Hz), 7.27 (t, 1H, $J=7.8$ Hz), 7.11 (d, 1H, $J=7.8$ Hz), 6.04 (s, 1H), 4.05 (s, 2H), 3.62 (apparent t, 2 H, $J=4.9$ Hz), 2.51 (m, 3H), 1.49 (s, 9H), 1.25 (d, 6H, $J=7.4$ Hz); ESMS m/e: 345.5 ($M + H$)⁺. Anal. Calc. for $C_{20}H_{28}N_2O_3+0.175 CHCl_3$: C, 66.33; H, 7.77; N, 7.67. Found: C, 66.20; H, 7.41; N, 7.88

TERT-BUTYL 4-[3-(ISOBUTYRYLAMINO)PHENYL]-1-PIPERIDINECARBOXYLATE: A mixture of 2.90 g (8.40 mmol) of tert-butyl 4-[3-(isobutyrylamino)phenyl]-3,6-dihydro-1(2H)-pyridinecarboxylate and 0.80 g of 10% yield Pd/C in 100 mL of ethanol was stirred under a hydrogen balloon for 24 hours. The reaction mixture was passed through a column of Celite 545 filtering agent, the filtrate was dried ($MgSO_4$) and concentrated *in vacuo*. The residue was purified by column chromatography (silica, 9.5 : 0.5 , dichloromethane : methanol + 1% isopropyl amine) to afford 2.40 g (84.0% yield) of the desired product: 1H NMR (400 MHz, $400 MHz, CDCl_3$) δ 7.49-7.44 (m, 2H), 7.24 (t, 1H, $J=7.6$ Hz), 6.93 (d, 1H, $J=7.6$ Hz), 4.20-4.10 (m, 2H), 2.86-2.45 (m, 4H), 1.86-1.75 (m, 4H), 1.48 (s, 9H), 1.24 (d, 6H, $J=6.8$ Hz); ESMS m/e : 345.2 ($M + H$)⁺; Anal. Calc. for $C_{20}H_{30}N_2O_3+0.3H_2O$: C,

68.27; H, 8.77; N, 7.96. Found: C, 68.25; H, 8.54; N, 7.84.

2-METHYL-N-[3-(4-PIPERIDINYL)PHENYL]PROPANAMIDE: Into a
5 stirred solution of 2.20 (6.50 mmol) of *tert*-butyl 4-[3-(isobutyrylamino)phenyl]-1-piperidinecarboxylate in 100
ml of 1,4-dioxane at 0 °C was bubbled HCl gas for 10
minutes. The reaction mixture was allowed to warm to
room temperature and the bubbling of the HCl gas was
10 continued for 1 hour. The solvent was removed *in vacuo*,
the residue was dissolved in 50 mL of water and was
neutralized by the addition of KOH pellets. The aqueous
solution was extracted with 3 X 80 mL of
dichloromethane, the combined organic extracts were
15 dried ($MgSO_4$), filtered and concentrated *in vacuo*. The
residue was purified by column chromatography (silica, 9 : 1 ,dichloromethane : methanol + 1% isopropyl amine) to
afford 0.700 g (46.0% yield) of the desired product: 1H
NMR (400 MHz, 400 MHz, $CDCl_3$) δ 7.47 (s, 1H), 7.40 (d,
20 1H, J=7.8 Hz), 7.24 (t, 1H, J=7.8 Hz), 7.00 (d, 1H,
J=7.8 Hz), 3.23-3.14 (m, 5H), 2.82-2.57 (m, 4H), 1.20
(d, 6H, J=6.8 Hz); ESMS m/e : 247.2 (M + H)⁺;
The hydrochloride salt was used for the combustion
analysis: Anal. Calc. for $C_{15}H_{22}N_2O+HCl+0.15 CHCl_3$: C,
25 60.51; H, 7.76; N, 9.32. Found: C, 60.57; H, 7.83; N,
8.88.

3-(4-PIPERIDINYL)ANILINE: 1H NMR (400 MHz, 400 MHz,
 $CDCl_3$) δ 7.01 (t, 1H, J=7.6 Hz), 6.62-6.54 (m, 3H), 3.16
30 (br d, 2H, J=10.3 Hz), 2.75 (dt, 2H, J=2.7, 12.3 Hz),
2.56 (tt, 1H, J=3.6, 12.3 Hz), 1.81 (br d, 2H, J=12.3
Hz), 1.65 (dq, 2H, J=4.0, 12.3 Hz); ESMS m/e : 177.2 (M
+ H)⁺.

TERT-BUTYL 4-(4-NITROPHENYL)-3,6-DIHYDRO-1(2H)-

PYRIDINECARBOXYLATE: To a 25-mL RB flask, equipped with a condensor, was added tert-butyl 4-

5 {[(trifluoromethyl)sulfonyl]oxy}-3,6-dihydro-1(2H)-
pyridinecarboxylate (1.0 g), 4-nitrophenylboronic acid
(0.71 g), sodium carbonate (0.430 mL of 2M solution),
lithium chloride (0.382 g),
10 tetrakis(triphenylphosphine)- palladium (0) (0.173 g)
and ethylene glycol dimethyl ether (10 mL). The
reaction mixture was flushed with Argon three times,
then the reaction mixture was heated to 100 °C for 3 hrs.
After cooling to room temperature, the reaction mixture
was diluted with methylene chloride (30 mL) and water
15 (30 mL) and the organic layer was separated. The
aqueous layer was extracted with methylene chloride
(3x20 mL) and the combined organic extracts were washed
with sat NH₄Cl (20 mL) and brine (20 mL), dried over
MgSO₄ and concentrated under reduced pressure. The
residue was purified by chromatography (6:1=hexane:ethyl
acetate with 1% NH₃) to afford the product (0.55 g,
20 59.9%) as a yellow oil. The compound is not stable at
room temperature and should be used as prompt as
practical: ¹H NMR (400 MHz, 400 MHz, CDCl₃) δ 8.20 (d,
25 2H, J=8.6 Hz), 7.51 (d, 2H, J=8.6 Hz), 6.24 (m, 1H),
4.13 (m, 2H), 3.67 (apparent t, 2H, J=5.5 Hz), 2.55 (m,
2H), 1.49 (s, 9H).

4-(4-NITROPHENYL)-1,2,3,6-TETRAHYDROPYRIDINE:

30 4-(4-Nitrophenyl)-1,2,3,6-tetrahydropyridine was
prepared by a similar procedure to that used for the
preparation of 2-methyl-N-[3-(4-
piperidinyl)phenyl]propanamide using HCl gas and *tert*-

Butyl 4-(4-Nitrophenyl)-3,6-dihydro-1(2H)-
pyridinecarboxylate (130 mg) in dioxane (5.0 mL) at room
temperature. The reaction mixture was concentrated in
vacuo to give the crude product (69.8 mg) that used in
5 the next reaction without further purification.

Oxazolidinone Intermediates:

AMINO-(3,5-DIFLUOROPHENYL)-ACETONITRILE.

10 Through a solution of 3,5-difluorobenzaldehyde (25.0 g,
0.176 mol) in MeOH (500 mL) in a round bottom flask, was
bubbled ammonia gas for two hours at room temperature.
The flask was then cooled to 0 °C and trimethylsilyl
cyanide was then added slowly. The reaction mixture was
15 stirred for 2 h, at which time TLC analysis indicated
that the reaction was complete ($R_f = 0.35$, 3:2
hexane/EtOAc). The solvent was removed in vacuo and the
residue was subjected to flash column chromatography on
silica gel to obtain the desired product.

20 **AMINO-(3,5-DIFLUOROPHENYL)-ACETIC ACID METHYL ESTER.**

Into a well-stirred solution of amino-(3,5-
difluorophenyl)-acetonitrile (22.0 g, 0.130 mol), a
solution of HCl in MeOH (200 mL) was added at room
25 temperature. The resulting yellow solution was stirred
at room temperature for 10 h and was heated at reflux
temperature for 1.5 h. After cooling, the solvent was
removed in vacuo and the resulting yellow solid was
dissolved in water (200 mL). The aqueous solution was
then carefully basified with 20% NaOH solution to pH 9.
30 The aqueous layer was extracted with CH_2Cl_2 (3 x 100 mL).
The organic layer was separated and dried over Na_2SO_4 ,
filtered and the solvent was removed in vacuo to obtain

the desired product which was used in the next step without purification.

2-AMINO-2-(3,5-DIFLUOROPHENYL)-ETHANOL.

5 Into a well-stirred suspension of LiAlH₄ (4.7 g, 0.125 mol) in THF (120 mL) in a 3-necked round bottom flask fitted with a condenser and a dropping funnel, was added a solution of amino-(3,5-difluorophenyl)-acetic acid methyl ester (10.0 g, 0.05 mol) in THF (100 mL) dropwise at 0 °C. The resulting greenish brown suspension was heated at reflux temperature for 2 h. The reaction mixture was cooled to 0 °C and then carefully quenched sequentially with 5 mL of water, 5 mL of 3N NaOH followed by 15 mL of water. The resulting suspension 10 was filtered through a fritted glass funnel. To the filter cake was added 100 mL Et₂O and the suspension was heated at reflux temperature for 20 min. The suspension 15 was filtered and the combined filtrates were dried over MgSO₄, filtered and the solvent was removed *in vacuo*. 2- Amino-2-(3,5-difluorophenyl)-ethanol was obtained as a 20 yellow glassy syrup which was used in the next step without further purification.

[1-(3,4-DIFLUOROPHENYL)-2-HYDROXY-ETHYL]-CARBAMIC ACID-TERT-BUTYL ESTER.

25 Into a solution of 2-amino-2-(3,4-difluorophenyl)-ethanol (8.6 g, 49.7 mmol) in CHCl₃ (150 mL) at 0 °C was added a solution of di-tert-butyl dicarbonate (11.4 g, 52.0 mmol) in CHCl₃ (50 mL) in one portion and the 30 resulting solution was stirred overnight at room temperature. The solvent was removed *in vacuo* and the residue was subjected to column chromatography on silica gel (2:1 hexane-EtOAc followed by EtOAc) to obtain [1-

(3,4-difluorophenyl)-2-hydroxy-ethyl]-carbamic acid-tert-butyl ester as a white solid (10.0 g, 74% yield).

(+)-4-(3,4-DIFLUOROPHENYL)-OXAZOLIDIN-2-ONE.

5 Into a well-stirred suspension of NaH (1.1 g, 45.8 mmol) in THF (40 mL) at R.T. was added a solution of [1-(3,4-difluorophenyl)-2-hydroxy-ethyl]-carbamic acid-tert-butyl ester (5.0 g, 18.3 mmol) in THF (20 mL) via a dropping funnel at room temperature. The resulting
10 suspension was stirred for 3 h and then quenched carefully with 10 mL of water. The biphasic mixture was extracted with 100 mL of Et₂O, washed with brine, filtered and the solvent was removed *in vacuo*. The gummy residue thus obtained was purified by column chromatography over silica gel (R_f = 0.15, 3:2 hexane-EtOAc) to obtain 4-(3,5-difluorophenyl)-oxazolidin-2-one
15 as a white flaky solid (2.8 g, 77% yield). M.P. 81-83 °C; ¹H NMR (300 MHz, CDCl₃) δ 4.13 (dd, J=6.6 Hz, J=8.7 Hz, 1 H), 4.73 (t, J=8.7 Hz, 1 H), 4.94 (dd, J=6.6 Hz, J=8.7 Hz, 1 H), 6.08 (br s, 1 H), 7.03-7.23 (m, 3 H).
20 The enantiomers were separated on a Chiralcel OD (20 x 250 mm) using 80% hexane/20% isopropyl alcohol as the eluting system at 12.0 mL/min (U.V. 254 nm). The retention times for the two isomers were 16.19 min and
25 20.08 min respectively.

4-NITROPHENYL (4S)-4-(3,4-DIFLUOROPHENYL)-2-OXO-1,3-

OXAZOLIDINE-3-CARBOXYLATE: Into a suspension of NaH (0.14 g, 5.30 mmol) in 20 mL of anhydrous THF under
30 argon, a solution of (+)-4-(3,5-difluorophenyl)-oxazolidin-2-one (0.88 g, 4.42 mmol) in THF was added dropwise (dropping funnel). The resulting suspension was stirred at room temperature for 30 min. This

suspension was then added dropwise via cannula into another round bottom flask containing a solution of 4-nitrophenylchloroformate (1.11 g, 5.30 mmol) in 25 mL of THF and cooled at -78 °C over a period of 15 min. The stirring was continued for 2 h after which the solvent was removed and the residue was purified by column chromatography on silica gel with 1:1 hexane/CH₂Cl₂ followed by CH₂Cl₂ (*R*_f= 0.4, CH₂Cl₂) to obtain the desired product as a white solid (1.55 g, 86% yield).

Similarly, following the above procedure, 4-(3,5-trifluorophenyl)-2-oxo-oxazolidine-3-carboxylic acid-4-nitro-phenyl ester and 4-(3,4,5-trifluorophenyl)-2-oxo-oxazolidine-3-carboxylic acid-4-nitro-phenyl ester were obtained. The oxazolidinone enantiomers were resolved on a chiracel OD column (as in the previous example) and the 4-nitro-phenyl esters were prepared using 4-nitrophenyl chloroformate.

4-NITROPHENYL (4S)-4-(3,5-DIFLUOROPHENYL)-2-OXO-1,3-OXAZOLIDINE-3-CARBOXYLATE: ¹H NMR (400 MHz, CDCl₃) δ 8.26 (d, 2H, J= 9.3 Hz), 7.33 - 6.81 (m, 5H), 5.41 (dd, 1H, J=4.1, 8.7 Hz), 4.81 (t, 1H, J=9.3 Hz), 4.33 (dd, 1H, J=4.1, 9.3 Hz); Anal. Calc. for C₁₆H₁₀F₂N₂O₆+0.2EtOAc: C, 52.84; H, 3.06; N, 7.34. Found: C, 53.26; H, 2.83; N, 7.73

4-NITROPHENYL (4S)-2-OXO-4-(3,4,5-TRIFLUOROPHENYL)-1,3-OXAZOLIDINE-3-CARBOXYLATE: ¹H NMR (400 MHz, CDCl₃) δ 8.27 (d, 2H, J=9.0 Hz), 7.31 (d, 2H, J=9.0 Hz), 7.11-7.02 (m, 2H), 5.37 (dd, 1H, J=4.1, 9.0 Hz), 4.81 (apparent t, 1H, J=9.0 Hz), 4.33 (dd, 1H, J=4.1, 9.0 Hz); Anal. Calc. for

C₁₆H₉F₃N₂O₆: C, 50.27; H, 2.37; N, 7.33. Found: C, 50.56; H, 2.50; N, 7.49.

1-(3,4-DIFLUOROPHENYL)-2-METHYL-2-HYDROXYPROPYLAMINE.

5 Into a well-stirred solution of methyl 2-amino-2-(3,4-difluorophenyl)acetate (10.5 g, 52.19 mmol) in anhydrous ether (200 mL) at 0 °C a solution of methylmagnesium bromide (3 M, 87 mL, 261 mmol) in ether was added over 10 minutes. The reaction mixture was stirred at 0 °C for 10 2.5 h and allowed to warm to room temperature. After 12 h, the reaction mixture was carefully poured onto a mixture of ice (300 g) and saturated aqueous ammonium chloride (50 g). The ether layer was separated and the aqueous layer was extracted with more ether (4 X 200 mL). The combined extracts were dried with magnesium sulfate and the solvent evaporated. The crude product was purified by column chromatography on silica gel using chloroform/methanol/2M ammonia in methanol (1000:20:10, 1000:40:20, 1000:80:40) as the eluent to give the product as an oil (6.5 g, 62% yield). The ¹H-NMR and MS confirmed this to be the desired product.

4-(3,4-DIFLUOROPHENYL)-5,5-DIMETHYL-2-OXO-OXAZOLIDINE.

A mixture of 1-(3,4-difluorophenyl)-2-methyl-2-hydroxypropylamine (3.00 g, 14.9 mmol) and carbonyldiimidazole (2.418 g, 14.9 mmol) in dichloromethane (150 mL) was heated at reflux temperature for 36 h and the solvent evaporated. The residue was purified by column chromatography on silica gel using chloroform/ethyl acetate (9:1) to give the product as a viscous oil which solidified on standing (1.80 g, 50% yield).

4-(3,4-DIFLUOROPHENYL)-5,5-DIMETHYL-2-OXO-3-(4-NITROPHENYLOXYCARBONYL)OXAZOLIDINE.

Into a stirred suspension of sodium hydride (60% suspension in paraffin 203 mg, 1.4 eq.) in THF (20 mL) at 0 °C, a solution of 4-(3,4-difluorophenyl)-5,5-dimethyl-2-oxo-oxazolidine (870 mg, 3.622 mmol) in THF (5 mL) was added followed by stirring for 30 minutes. This suspension was added to a solution of 4-nitrophenyl chloroformate (950 mg, 4.71 mmol) in THF (20 mL) at -78 °C under argon and the stirring was continued for 2 h. It was slowly warmed to room temperature and after 4 h the solvent was evaporated. The residue was mixed with dichloromethane (150 mL), washed with 0.05 N sodium hydroxide (3 X 10 mL), and dried (sodium sulfate). The solvent was evaporated and the residue was purified by column chromatography on silica gel using chloroform/ethyl acetate (9:1) as the eluent to give the product as a white powder (860 mg, 59% yield).

4-NITROPHENYL 4-(3,4-DIFLUOROPHENYL)-5,5-DIMETHYL-2-OXO-1,3-OXAZOLIDINE-3-CARBOXYLATE: ^1H NMR (400 MHz, CDCl_3) δ 8.24 (d, 2H, $J=9$ Hz), 7.29 – 6.97 (m, 5H), 5.04 (s, 1H), 1.09 (s, 6H); Anal. Calc. for $\text{C}_{18}\text{H}_{14}\text{F}_2\text{N}_2\text{O}_6+0.2\%$ H_2O : C, 54.61; H, 3.67; N, 7.08. Found: C, 54.89; H, 3.59; N, 7.41.

a. BENZHYDRYLINDENE-(3,4-DIFLUOROBENZYL)-AMINE

Into a solution of 3,4-difluorobenzylamine (9.8 g, 69 mmol) and benzophenone (13.0 g, 71.0 mmol) in toluene (200 mL) was added a catalytic amount of $\text{BF}_3\cdot\text{OEt}_2$ and the resulting solution was heated at reflux temperature for 12 h. The reaction mixture was concentrated *in vacuo*, yielding an oil (21 g, >95%), which was characterized by

NMR analysis and subjected to the following reaction without any further purification. ^1H NMR (CDCl_3) δ 4.57 (s, 2H), 7.80-6.80 (m, 13H).

5 **b. 1-(BENZHYDRYLIDEN-AMINO)-1-(3,4-DIFLUORO-PHENYL)-
PROPAN-2-OL.**

10 Into a solution of the benzhydrylindene-(3,4-difluoro-benzyl)-amine (21 g, 69 mmol) in 250 ml of dry THF was added *tert*-butyllithium (1.7 M, 60 ml) dropwise and the resulting solution was stirred at -78 °C for 0.5 h. To the solution was added acetaldehyde (10 ml, 180 mmol) in 100 ml of THF and the solution was stirred at -78 °C for 2 h and 25 °C for 1 h. The reaction mixture was quenched by addition of brine. The reaction mixture was diluted with 500 ml of Et_2O and washed with brine. The organic layer was dried over Na_2SO_4 and concentrated *in vacuo* to give an oil, which was taken to the next step without any further purification. ^1H NMR (CDCl_3) δ 1.04 (d, 3H), 2.77 (broad s. 1H), 4.08(m, 1H), 4.15 (d, 1H), 7.80-6.80 (m, 13H).

15 **c. 1-AMINO-1-(3,4-DIFLUORO-PHENYL)-PROPAN-2-OL**
A solution of crude product from the previous procedure and $\text{MeONH}_2\cdot\text{HCl}$ (10 g, 120 mmol) was diluted in 200 ml of MeOH and stirred for 12 h. The reaction mixture was concentrated *in vacuo*, yielding an oily residue, which was re-dissolved in 200 ml of EtOAc and washed with brine. The organic layer was concentrated *in vacuo* to produce an oily mixture, which was subjected to column chromatography (5% NH_3 saturated MeOH/ CHCl_3) to yield the desired product (8.8 g, 68% yield from 3,4-difluorobenzylamine) as a mixture of diastereomers. ^1H NMR (CDCl_3) (~ 4:1 mixture of the diastereomers) δ 1.02

(d, $J=6.0$ Hz, 3 H), 1.04 (d, $J=6.3$ Hz, 3 H), 2.10 (br, 6 H), 3.56-3.69 (m, 2 H), 3.88-3.92 (m, 2 H), 7.02-7.17 (m, 6 H).

5 **d. [1-(3,4-DIFLUOROPHENYL)-2-HYDROXY-PROPYL]-CARBAMIC
ACID-TERT-BUTYL ESTER**

10 Into a solution of 1-amino-1-(3,4-difluorophenyl)-propan-2-ol (13.1 g, 70.1 mmol) in CHCl_3 (150 mL) at 0 °C was added a solution of di-tert-butyl dicarbonate (19.3 g, 87.6 mmol) in CHCl_3 (50 mL) in one portion and the resulting solution was stirred overnight at room temperature. The solvent was removed *in vacuo* and the residue was subjected to column chromatography on silica gel (2:1 hexane-EtOAc followed by EtOAc) to obtain [1-(3,4-difluorophenyl)-2-hydroxy-propyl]-carbamic acid-tert-butyl ester as a viscous oil (18.4 g, 91% yield). ^1H NMR (CDCl_3) (~ 4:1 mixture of the diastereomers) δ 1.05 (d, $J=6.6$ Hz, 3 H), 1.25 (d, $J=6.0$ Hz, 3 H), 1.41 (br, 20 H), 3.92-4.19 (br, 2 H), 4.45-4.60 (m, 2 H), 5.41-5.49 (br, 2 H), 7.02-7.17 (m, 6 H).

15 **e. 4-(3,4-DIFLUOROPHENYL)-5-METHYL-OXAZOLIDIN-2-ONE**

20 Into a well-stirred solution of [1-(3,4-difluorophenyl)-2-hydroxy-propyl]-carbamic acid-tert-butyl ester (0.43 g, 1.5 mmol) THF (20 mL) was added 95% NaH (0.09 g, 3.8 mmol) at room temperature. When the reaction was carried out on a larger (> 5 g) scale, 1.0 equivalent of KH and 1.5 eq. of NaH was used as the base. The resulting suspension was stirred for 3 h at about 35 °C (warm water bath) and then quenched carefully with ice. The biphasic mixture was extracted with 100 mL of EtOAc, washed with brine, dried over Na_2SO_4 , filtered and the solvent was removed *in vacuo*. The two diastereomers

were separated by column chromatography over silica gel
(First isomer: 0.16 g, R_f = 0.6, 3:1 hexane-EtOAc; second
isomer: 0.18 g, R_f = 0.5, 3:1 hexane-EtOAc). NOE
experiment suggested that the first diastereomer had the
5 methyl and the aryl group in trans configuration while
the second diastereomer had cis relationship between the
two groups.

The ^1H NMR spectrum for the *trans* diastereomers is as
follows. ^1H NMR (CDCl_3) δ 1.49 (d, J = 6.0 Hz, 3H), 4.37
10 (dq, J = 6.0 Hz, J = 7.2 Hz, 1H), 4.45 (d, J = 7.2 Hz,
1H), 6.63 (br s, 1H), 7.08-7.28 (m, 3H).

The ^1H NMR spectrum for the *cis* diastereomers is as
follows. ^1H NMR (CDCl_3) δ 0.96 (d, J = 6.6 Hz, 3H), 4.91
15 (d, J = 8.1 Hz, 1H), 4.99 (dq, J = 6.6 Hz, J = 8.1 Hz,
1H), 6.63 (br s, 1H), 7.08-7.28 (m, 3H).

Enantiomers of the diastereomers were separated by HPLC
by using a Chiralcel OD column (20 x 250 mm) with 80%
20 hexane/20% isopropyl alcohol/ 0.1 % diethylamine as the
eluting system (12 mL/min) under isocratic conditions
(U.V. 254 nm).

f. 4-(3,4-DIFLUOROPHENYL)-5-METHYL-2-OXO-OXAZOLIDINE-3-CARBOXYLIC ACID-4-NITRO-PHENYL ESTER

25 Into a solution of 4-(3,4-difluorophenyl)-5-methyl-
oxazolidin-2-one (0.97 g, 4.55 mmol) in 60 mL THF was
added a solution of n-butyllithium in hexane (3.06 mmol,
4.9 mmol) dropwise via a syringe under argon atmosphere
30 at -78 °C. The resulting yellow solution was stirred at
-78 °C for 40 min. This solution was then added dropwise
via a cannula into another round bottom flask containing
a solution of 4-nitrophenylchloroformate (1.03 g, 5.1

mmol) in 60 mL of THF, cooled at -78 °C, over a period of 15 min. After five minutes, the flask was removed from the cooling bath and stirring was continued for 1 h. The reaction mixture was quenched by adding ice and it was extracted with EtOAc. The organic extracts were washed with brine and the organic layer was dried over Na₂SO₄. The solvent was removed after filtration and the residue was purified by column chromatography on silica gel with 1:1 hexane/CH₂Cl₂ followed by CH₂Cl₂ ($R_f = 0.4$, CH₂Cl₂) to give the desired product.

The relative configurations of the cis and trans isomers were assigned on the basis of ¹H NMR analysis of the respective p-nitrophenyloxycarbonyl derivatives. For the trans isomer, an NOE was observed between the 15 protons of the C-5 methyl group and the proton at C-4. No NOE was observed between the protons at the C-4 and C-5 positions of this isomer, which was thus assigned *trans* stereochemistry. For the cis isomer, no NOE was observed between the protons of the C-5 methyl group and the proton at C-4. However, a NOE was observed between the protons at the C-4 and C-5 positions, leading us to assign this isomer *cis* stereochemistry. The vicinal coupling constants of the C-4 protons of *cis* ($J = 7.8$ Hz) and *trans* ($J = 5.1$ Hz) are also consistent with the 20 values reported for similar oxazolidinones, and were thus helpful in making the stereochemical assignments (Dondoni, A.; Perrone, D.; Semola, T. *Synthesis* **1995**, 25 181).

In order to assign the absolute configurations at the stereogenic centers of the oxazolidinone rings, a new synthetic route was designed which employed an enantiomerically pure substrate derived from the chiral 30

pool. Commercially available (*S*)-(+)-methyl lactate was converted into its pyrrolidine amide according to the method of Martin et al (Martin, R.; Pascual, O.; Romea, P.; Rovira, R.; Urpi, F.; Vilarrasa, J. *Tetrahedron Lett.* **1997**, *38*, 1633). Following the protection of the hydroxy group of (2*S*)-1-oxo-1-(1-pyrrolidinyl)-2-propanol to a TBDMS group, treatment of tert-butyl(dimethyl)silyl (1*S*)-1-methyl-2-oxo-2-(1-pyrrolidinyl)ethyl ether with 3,4-difluorophenyllithium yielded (2*S*)-2-{[tert-butyl(dimethyl)silyl]oxy}-1-(3,4-difluorophenyl)-1-propanone as the sole product, which was then converted to (2*S*)-2-{[tert-butyl(dimethyl)silyl]oxy}-1-(3,4-difluorophenyl)-1-propanone oxime. Reduction of the (2*S*)-2-{[tert-butyl(dimethyl)silyl]oxy}-1-(3,4-difluorophenyl)-1-propanone oxime with LiAlH₄, N-acylation, and base induced cyclization provided oxazolidinone diastereomers, which were separated by flash column chromatography. The enantiomeric purity of these isomers was confirmed by chiral HPLC analysis and their relative configurations were assigned by comparison of their ¹H NMR spectra with those of the racemic isomers. As the absolute configuration at C-5 of the lactic acid derived oxazolidinone described above is (*S*), the C-4 center in *trans* compounds also has the (*S*) configuration. Accordingly, the absolute configurations for the stereogenic centers in the *cis* compounds are assigned accordingly (4*R*, 5*S*).

30 **4-NITROPHENYL (4S,5R)-4-(3,4-DIFLUOROPHENYL)-5-METHYL-2-OXO-1,3-OXAZOLIDINE-3-CARBOXYLATE:** ¹H NMR (400 MHz, CDCl₃) δ 8.25 (d, 2H, J=8.8 Hz), 7.30 - 6.99 (m, 5H), 5.35 (d, 1H, J=7.7 Hz), 5.07 (apparent quintet, 1H),

1.17 (d, 3H, J=6.5 Hz); Anal. Calc. for
 $C_{17}H_{12}F_2N_2O_6 + 0.5H_2O$: C, 52.72; H, 3.38; N, 7.23. Found: C,
53.09; H, 3.19; N, 7.50.

5 **(+)-2-AMINO-3-(3,4-DIFLUORO)-PHENYL-PROPAN-1-OL:** (+)-
3,4-difluorophenyl alanine (1.0 g, 5.0 mmol) was added
in small portions to a stirring suspension of LiAlH₄
(0.480 g, 12.5 mmol) in THF (30 mL) at 0 °C. The
resulting gray suspension was then heated at reflux for
10 2 h. The reaction mixture was cooled to 0 °C and then
carefully quenched sequentially with water (0.5 mL), 3 N
NaOH (0.5 mL), and water (1.50 mL). The resulting
suspension was filtered through a fritted glass funnel.
Ether (50 mL) was added to the filter cake and the
suspension was heated at reflux temperature for 20 min.
15 The suspension was filtered and was combined with the
previous filtrate. The combined organics were dried
over MgSO₄, filtered and the solvent was removed in
vacuo. 2-Amino-3-(3,4-difluoro)-phenyl-propan-1-ol was
20 obtained as a white solid (0.500 g, 100%) which was used
in the next step without further purification.

25 **(+)-[1-(3,4-DIFLUOROBENZYL)-2-HYDROXY-ETHYL]-CARBAMIC
ACID-TERT-BUTYL ESTER:** A solution of di-tert-butyl
dicarbonate (0.640 g, 2.90 mmol) in CHCl₃ (10 mL) was
added in one portion to a solution of (+)-2-amino-3-(3,4-difluoro)-phenyl-propan-1-ol (0.500 g, 2.62 mmol)
in CHCl₃ (20 mL) at 0 °C and the resulting solution was
stirred overnight at room temperature. The solvent was
30 removed in vacuo and the residue was chromatographed
(2:1 hexane-EtOAc, followed by EtOAc), giving (+)-[1-(3,4-difluorobenzyl)-2-hydroxy-ethyl]-carbamic acid-
tert-butyl ester as a white solid (0.640 g, 99%).

(+)-4-(3,4-DIFLUORO-BENZYL)-OXAZOLIDIN-2-ONE: A solution of (+)-[1-(3,4-difluorobenzyl)-2-hydroxy-ethyl]-carbamic acid-tert-butyl ester (1.00 g, 4.00 mmol) in THF (10 mL) was added via a dropping funnel to a stirring suspension of 95% NaH (0.12 g, 5.0 mmol) in THF (20 mL) at room temperature. The resulting suspension was stirred for 3 h and then quenched carefully with water (10 mL). The biphasic mixture was extracted with Et₂O (50 mL), washed with brine, filtered and the solvent was removed *in vacuo*. The resulting gummy residue was purified by column chromatography (R_f = 0.25, 3:2 hexane-EtOAc), to give the desired product as a white solid (0.320 g, 76%).

15

(+)-4-(3,4-DIFLUORO-BENZYL)-OXAZOLIDIN-2-ONE-3-CARBOXYLIC ACID-4-NITRO-PHENYL ESTER: A solution of (+)-4-(3,4-difluoro-benzyl)-oxazolidin-2-one (0.210 g, 1.0 mmol) in THF (10 mL) was added dropwise via a dropping funnel to a stirring suspension of NaH (30.0 mg, 1.30 mmol) in anhydrous THF (10 mL) under argon. The resulting suspension was stirred at room temperature for 30 min. This suspension was then added dropwise via cannula to a solution of 4-nitrophenylchloroformate (0.300 g, 1.50 mmol) in THF (20 mL) at -78 °C over 15 min. Stirring was continued for 2 h after which the solvent was removed and the residue was purified by column chromatography (1:1 hexane/CH₂Cl₂, followed by CH₂Cl₂; R_f = 0.4, CH₂Cl₂), to give the desired product as a yellow solid (0.350 g, 82%).

Similarly, 4-nitrophenyl 4-(4-fluorobenzyl)-2-oxo-1,3-oxazolidine-3-carboxylate was obtained from the corresponding p-fluorophenyl alanine:

5 **4-NITROPHENYL 4-(4-FLUOROBENZYL)-2-OXO-1,3-OXAZOLIDINE-3-CARBOXYLATE:** ^1H NMR (400 MHz, CDCl_3) δ 8.32 (d, 2H, $J=9.3$ Hz), 7.42 (d, 2H, $J=8.9$ Hz), 7.24-6.99 (m, 4H), 4.69 - 4.59 (m, 1H), 4.35 (t, 1H, $J=8.6$ Hz), 4.23 (dd, 1H, $J=2.7, 9.3$ Hz), 3.37 (dd, 1H, $J=3.8, 13.6$ Hz), 2.94 (dd, 1H, $J=9.3, 13.6$ Hz); Anal. Calc. for $\text{C}_{17}\text{H}_{13}\text{FN}_2\text{O}_6$: C, 56.67; H, 3.64; N, 7.77. Found: C, 56.94; H, 3.76; N, 7.71.

15 **2-[6-(4-PHENYL-1-PIPERIDINYL)HEXYL]-1H-ISOINDOLE-1,3(2H)-DIONE:** To the 500 ml RB-flask was added 4-phenylpiperidine hydrochloride (5 g, 25 mmol), N-(6-bromohexyl)phthalimide (15.5 g, 50 mmol), N,N-diisopropylethylamine (21.8 ml, 125 mmol), tetrabutylammonium iodide (0.2 g), and dioxane (250 ml) at room temperature. The reaction mixture was stirred at 100 oC for 72 h. The solvent was removed *in vacuo* and the crude product was purified by flash chromatography (98:2 = Chloroform : 2N ammonia in methanol) to afford 7.67 g of the desired product (77% yield): ^1H NMR (400 MHz, CDCl_3) δ 7.78-7.79 (m, 2H), 7.74-7.65 (m, 2H), 7.32-7.14 (m, 5H), 3.69 (t, 2H, $J=7.35$ Hz), 3.06 (d, 2H, $J=11.0$ Hz), 2.49 (quintet, 1H, $J=7.6$ Hz), 2.36 (t, 2H, $J=7.6$ Hz), 2.02 (t, 2H, $J=12.5$ Hz), 1.82 (br s, 4H), 1.69 (t, 2H, $J=6.3$ Hz), 1.54 (br s, 2H), 1.37 (br s, 4H); ESMS m/e: 391.3 ($\text{M} + \text{H})^+$; Anal. Calc. for $\text{C}_{25}\text{H}_{30}\text{N}_2\text{O}_2 + 0.2\text{H}_2\text{O}$: C, 76.19; H, 7.77; N, 7.11. Found: C, 76.14; H, 7.38; N, 7.13.

General procedure for the Preparation of the substituted
4-[4-(3-aminophenyl)-1-piperidinyl]-1-(phenyl)-1-
butanones:

A mixture of 4-(3-aminophenyl)piperidine (2.0 mmol), 2.4
5 mmol of the appropriate substituted phenyl butyryl
chloride, 3.0 mmol of K_2CO_3 , and 10 mg of 18-crown-6 in 5
mL of toluene were heated at 110 °C for 2.5 days. The
reaction mixture was concentrated and chromatographed on
silica (5% methanol in dichloromethane) to give the
10 desired compound:

**4-[4-(3-AMINOPHENYL)-1-PIPERIDINYL]-1-(4-PHOXYPHENYL)-
1-BUTANONE:** 305 mg; ESMS m/e : 415.4 ($M + H$)⁺.

15 **4-[4-(3-AMINOPHENYL)-1-PIPERIDINYL]-1-(4-CHLOROPHENYL)-
1-BUTANONE:** 500 mg; Anal. Calc for $C_{21}H_{25}ClN_2O+0.3H_2O$: C,
69.62; H, 7.12; N, 7.73. Found: C, 69.63; H, 7.34; N,
7.60; ESMS m/e : 357.3 ($M + H$)⁺.

20 **4-[4-(3-AMINOPHENYL)-1-PIPERIDINYL]-1-PHENYL-1-BUTANONE:**
250 mg; Anal. Calc for $C_{21}H_{26}N_2O+0.2H_2O$: C, 77.36; H,
8.16; N, 8.59. Found: C, 77.55; H, 8.12; N, 8.75; ESMS
m/e : 323.3 ($M + H$)⁺

25 **4-[4-(3-AMINOPHENYL)-1-PIPERIDINYL]-1-(2,4-
DIMETHOXYPHENYL)-1-BUTANONE:** 330 mg; Anal. Calc for
 $C_{23}H_{30}N_2O_3+0.5H_2O$: C, 70.56; H, 7.98; N, 7.16. Found: C,
70.69; H, 7.87; N, 6.99; ESMS m/e : 383.3 ($M + H$)⁺

30 **General Procedure for the Acylation or Sulfenylation of
the Substituted 4-[4-(3-Aminophenyl)-1-piperidinyl]-1-
(4-phenyl)-1-butanones:**

A mixture of 1 equivalent of a substituted 4-[4-(3-aminophenyl)-1-piperidinyl]-1-(4-phenyl)-1-butanone, 1.5 equivalent of an acid chloride or a sulfonyl chloride, and 5 equivalents of diisopropylethylamine, in
5 dichloromethane was stirred at room temperature for two days. The reaction mixture was applied to a preparative TLC plate and eluted with dichloromethane: methanol (15:1, containing 1% isopropyl amine) to give the desired product.

10

General procedure for the Preparation of the substituted 4-N-(3-{1-[4-(phenyl)-4-oxobutyl]-4-piperidinyl}phenyl)acetamides:

A mixture of N-[3-(4-piperidinyl)phenyl]acetamide (1.0 eq) and an aryl substituted chlorobutyrophenone (2.0 eq), K₂CO₃ (5.0 eq), diisopropylethylamine (3.0 eq) and tetrabutylammonium iodide (cat. 5-10%) in dioxane (0.5 to 1.0 M) were heated at reflux temperature for 16 h. The reaction mixture was filtered and concentrated *in vacuo*. The crude product was chromatographed using silica preparative TLC (chloroform : methanol containing 0.5% isopropyl amine) to give the desired product.

25

Example 57
N-(3-{1-[4-(3,4-DIMETHYLPHENYL)-4-OXOBUTYL]-4-PIPERIDINYL}PHENYL)ACETAMIDE: ¹H NMR (CDCl₃) δ 7.75 (s, 1H), 7.71 (d, 1H, J=7.6 Hz), 7.45 (d, 2H, J=7.2 Hz), 7.35 (s, 1H), 7.26-7.22 (m, 2H), 6.93 (d, 1H, J=7.6 Hz), 3.24-3.21 (m, 2H), 3.04 (t, 2H, J=7.0 Hz), 2.67-2.63 (m, 2H), 2.59-2.48 (m, 1H), 2.32 (s, 6H), 2.30-2.27 (m, 2H), 2.18 (s, 3H), 2.14-2.06 (m, 2H), 2.00-1.80 (m, 4H); ESMS m/e : 393.3 (M + H)⁺.

Example 58

N- (3- {1- [4- (3, 4-DIMETHYLPHENYL) -4-OXOBUTYL] -4-
PIPERIDINYLPHENYL} PHENYL) -2-METHYLPROPANAMIDE: A mixture of
0.0500 g (0.200 mmol) of 2-methyl-N- [3- (4-
5 piperidinyl)phenyl]propanamide, 0.100 g (0.480 mmol) of
4-chloro-3', 4'-dimethylbutyrophene, 0.080 g (0.600
mmol) of K₂CO₃ and 0.090 g (0.600 mmol) of NaI in 5 mL of
DMF was heated at reflux temperature for 18 hours. The
reaction mixture was filtered, the filtrate was poured
10 into 5 mL of water and washed with 3 X 5 mL of ethyl
acetate. The combined organic extracts were dried
(MgSO₄), concentrated *in vacuo* and purified by
preparative TLC (silica; 9.5 : 0.5, dichloromethane :
methanol + 1% isopropyl amine) to afford 0.067 g (80.0%
15 yield) of the desired product: ¹H NMR (400 MHz, CDCl₃) δ
7.72 (d, 1H, J=8.0 Hz), 7.44 (s, 1H), 7.38 (d, 1H, J=8.0
Hz), 7.23-7.20 (m, 2H), 7.16 (s, 1H), 6.95 (d, 1H, J=6.8
Hz), 3.13-3.11 (m, 2H), 3.02 (t, 2H, J=7.0 Hz), 2.56-
2.40 (m, 4H), 2.32 (s, 6H), 2.17-2.15 (m, 2H), 2.04-1.78
20 (m, 6H), 1.25 (d, 6H, J=6.8 Hz); ESMS m/e : 421.3 (M +
H)⁺.

Example 59

N- (3- {1- [4- (3, 4-DIMETHYLPHENYL) -4-OXOBUTYL] -4-
25 PIPERIDINYLPHENYL} CYCLOHEXANE CARBOXAMIDE: ¹H NMR (400
MHz, CDCl₃) δ 7.80-6.81 (m, 7H), 3.41-3.00 (m, 4H), 2.95-
2.41 (m, 4H), 2.32 (s, 6H), 2.22-1.05 (m, 18H); ESMS m/e
: 461.4 (M + H)⁺.

30 **Example 60**

N- (3- {1- [4- (3, 4-DIMETHYLPHENYL) -4-OXOBUTYL] -4-
PIPERIDINYLPHENYL} PHENYL) -2-PHENYLACETAMIDE: ¹H NMR (400 MHz,
CDCl₃) δ 7.85-7.65 (m, 2H), 7.45-6.92 (m, 10H), 3.76 (s,

2H), 3.10-2.90 (m, 4H), 2.50-2.35 (m, 3H), 2.32 (s, 6H),
2.10-1.85 (m, 4H), 1.80-1.60 (m, 4H); ESMS m/e : 469.4
(M + H)⁺.

5 **Example 61**

N- (3-{1-[4-(3,4-DIMETHYLPHENYL)-4-OXOBUTYL]-4-PIPERIDINYL}PHENYL)-2-(3-METHOXYPHENYL)ACETAMIDE: ¹H NMR (400 MHz, CDCl₃) δ 7.76-7.65 (m, 2H), 7.38-7.12 (m, 6H), 6.95-6.80 (m, 3H), 3.82 (s, 3H), 3.70 (s, 2H), 3.10-2.90 (m, 4H), 2.50-2.38 (m, 3H), 2.32 (s, 6H), 2.10-1.85 (m, 4H), 1.80 -1.60 (m, 4H); ESMS m/e : 499.4 (M + H)⁺.

10 **Example 62**

N- (3-{1-[4-(3,4-DIMETHYLPHENYL)-4-OXOBUTYL]-4-PIPERIDINYL}PHENYL)-2-METHOXYACETAMIDE: ¹H NMR (400 MHz, CDCl₃) δ 7.80-7.75 (m, 2H), 7.50-7.38 (m, 2H), 7.34-6.90 (m, 3H), 4.00 (s, 2H), 3.51 (s, 3H), 3.30-2.95 (m, 4H), 2.70-2.50 (m, 3H), 2.32 (s, 6H), 2.15 -1.80 (m, 8H); ESMS m/e : 423.3 (M + H)⁺.

20

Example 63

N- (3-{1-[4-(3,4-DIMETHYLPHENYL)-4-OXOBUTYL]-4-PIPERIDINYL}PHENYL)METHANESULFONAMIDE: ¹H NMR (400 MHz, CDCl₃) δ 7.82-7.10 (m, 7H), 3.41 (s, 3H), 3.40-2.85 (m, 4H), 2.82-2.35 (m, 5H), 2.32 (s, 6H), 2.22-1.80 (m, 6H); ESMS m/e : 429.3 (M + H)⁺.

Example 64

N- (3-{1-[4-(3,4-DIMETHYLPHENYL)-4-OXOBUTYL]-4-PIPERIDINYL}PHENYL)ETHANESULFONAMIDE: ¹H NMR (400 MHz, CDCl₃) δ 7.75 (s, 1H), 7.71 (d, 1H, J=7.6 Hz), 7.30-7.09 (m, 4H), 7.02 (d, 1H, J=7.2 Hz), 3.36-3.05 (m, 6H),

2.77-2.52 (m, 3H), 2.32 (s, 6H), 2.15-1.82 (m, 8H), 1.37 (t, 3H, J=7.4 Hz); ESMS m/e : 443.3 (M + H)⁺

Example 65

5 N- (3-{1-[4-(4-CHLOROPHENYL)-4-OXOBUTYL]-4-

PIPERIDINYLPHENYL)ACETAMIDE: ¹H NMR (400 MHz, CDCl₃) δ 7.92 (d, 2H, J=8.8 Hz), 7.55-7.40 (m, 3H), 7.35 (s, 1H), 7.22 (t, 1H, J=8.0 Hz), 6.92 (d, 1H, J=8.0 Hz), 3.30-3.27 (m, 2H), 3.09 (t, 2H, J=7.0 Hz), 2.76-2.39 (m, 5H), 2.20 (s, 3H), 2.17-1.85 (m, 6H); ESMS m/e : 399.3 (M + H)⁺.

Example 66

15 N- (3-{1-[4-(4-CHLOROPHENYL)-4-OXOBUTYL]-4-

PIPERIDINYLPHENYL)-2-METHYLPROPANAMIDE: ¹H NMR (400 MHz, CDCl₃) δ 7.93 (d, 2H, J=8.6 Hz), 7.45 (d, 2H, J=8.6 Hz), 7.39 (d, 1H, J=7.2 Hz), 7.32 (s, 1H), 7.24 (t, 1H, J=7.8 Hz), 6.94 (d, 1H, J=8.4 Hz), 3.21-3.18 (m, 2H), 3.05 (t, 2H, J=7.0 Hz), 2.64-2.51 (m, 4H), 2.28-1.86 (m, 8H), 1.26 (d, 6H, J=6.8 Hz); ESMS m/e : 427.3 (M + H)⁺.

Example 67

25 N- (3-{1-[4-(4-CHLOROPHENYL)-4-OXOBUTYL]-4-

PIPERIDINYLPHENYL)CYCLOHEXANE CARBOXAMIDE: ¹H NMR (400 MHz, CDCl₃) δ 7.93 (d, 2H, J=8.4 Hz), 7.55-7.19 (m, 5H), 6.93 (d, 1H, J=7.6 Hz), 3.25-3.00 (m, 4H), 2.65-2.45 (m, 4H), 2.30-1.50 (m, 18H); ESMS m/e : 467.3 (M + H)⁺.

Example 68

30 N- (3-{1-[4-(4-CHLOROPHENYL)-4-OXOBUTYL]-4-

PIPERIDINYLPHENYL)-2-PHENYLACETAMIDE: ¹H NMR (400 MHz, CDCl₃) δ 7.92 (d, 2H, J=8.4 Hz), 7.46-7.26 (m, 9H), 7.20 (t, 1H, J=7.6 Hz), 6.92 (d, 1H, J=7.6 Hz), 3.75 (s, 2H),

3.15-3.13 (m, 2H), 3.03 (t, 2H, J=7.0 Hz), 2.64-2.46 (m, 3H), 2.22-1.60 (m, 8H); ESMS m/e : 475.3 (M + H)⁺.

Example 69

5 N- (3-{1-[4-(4-CHLOROPHENYL)-4-OXOBUTYL]-4-
PIPERIDINYL}PHENYL)-2-(3-METHOXYPHENYL)ACETAMIDE: ¹H NMR
(400 MHz, CDCl₃) δ 7.92 (d, 2H, J=8.4 Hz), 7.44 (d, 2H,
J=8.4 Hz) 7.38 (s, 1H), 7.35-7.25 (m, 3H), 7.19 (t, 1H,
J=7.8 Hz), 6.94-6.86 (m, 3H), 3.81 (s, 3H), 3.72 (s,
10 2H), 3.12-3.09 (m, 2H), 3.02 (t, 2H, J=6.8 Hz), 2.57-
2.44 (m, 3H), 2.20-1.60 (m, 8H); ESMS m/e : 505.3 (M +
H)⁺.

Example 70

15 N- (3-{1-[4-(4-CHLOROPHENYL)-4-OXOBUTYL]-4-
PIPERIDINYL}PHENYL)-2-METHOXYACETAMIDE: ¹H NMR (400 MHz,
CDCl₃) δ 7.93 (d, 2H, J=8.4 Hz), 7.50-7.25 (m, 5H), 6.98
(d, 1H, J=7.8 Hz), 4.01 (s, 2H), 3.57 (s, 3H), 3.30-3.15
(m, 2H), 3.06 (t, 2H, J=6.8 Hz), 2.70-2.50 (m, 3H),
20 2.35-1.80 (m, 8H); ESMS m/e : 429.3 (M + H)⁺.

Example 71

N- (3-{1-[4-(4-CHLOROPHENYL)-4-OXOBUTYL]-4-
PIPERIDINYL}PHENYL)METHANESULFONAMIDE: ¹H NMR (400 MHz,
CDCl₃) δ 7.95-6.96 (m, 8H), 3.48 (s, 3H), 3.28-2.90 (m,
25 6H), 2.80-2.57 (m, 3H), 2.38-1.86 (m, 6H); ESMS m/e :
435.2 (M + H)⁺.

Example 72

30 N- (3-{1-[4-(4-CHLOROPHENYL)-4-OXOBUTYL]-4-
PIPERIDINYL}PHENYL)ETHANESULFONAMIDE: ¹H NMR (400 MHz,
CDCl₃) δ 7.93 (d, 2H, J=8.2 Hz), 7.45 (d, 2H, J=8.2 Hz),
7.30-7.08 (m, 3H), 6.99 (d, 1H, J=7.6 Hz), 3.26-3.02 (m,

6H), 2.69-2.45 (m, 3H), 2.32-1.75 (m, 8H), 1.36 (t, 3H, J=7.4 Hz); ESMS m/e : 449.3 (M + H)⁺.

Example 73

5 N-{3-[1-(4-OXO-4-PHENYLBUTYL)-4-

PIPERIDINYL]PHENYL}ACETAMIDE: ¹H NMR (400 MHz, CDCl₃) δ 8.10-6.80 (m, 9H), 3.40-2.95 (m, 4H), 2.85-2.20 (m, 3H), 2.19 (s, 3H), 2.15-1.70 (m, 8H); ESMS m/e : 365.3 (M + H)⁺.

10

Example 74

2-METHYL-N-{3-[1-(4-OXO-4-PHENYLBUTYL)-4-

15

PIPERIDINYL]PHENYL}PROPANAMIDE: ¹H NMR (400 MHz, CDCl₃) δ 7.99 (d, 2H, J=7.4 Hz), 7.57 (t, 1H, J=7.4 Hz), 7.48 (t, 2H, J=7.4 Hz), 7.45-7.20 (m, 2H), 7.24 (t, 1H, J=8.0 Hz), 6.94 (d, 1H, 8.0 Hz), 3.24-3.21 (m, 2H), 3.09 (t, 2H, J=7.0 Hz), 2.57-2.25 (m, 4H), 2.31-1.84 (m, 8H), 1.26 (d, 6H, J=7.2 Hz); ESMS m/e : 393.3 (M + H)⁺.

20

Example 75

N-{3-[1-(4-OXO-4-PHENYLBUTYL)-4-PIPERIDINYL]PHENYL}-2-

25

PHENYLACETAMIDE: ¹H NMR (400 MHz, CDCl₃) δ 7.98 (d, 2H, J=7.6 Hz), 7.65-7.15 (m, 11H), 6.92 (d, 2H, J=7.2 Hz), 3.74 (s, 2H), 3.20-2.95 (m, 4H), 2.65-2.40 (m, 3H), 2.25-1.70 (m, 8H); ESMS m/e : 441.3 (M + H)⁺.

Example 76

2-(3-METHOXYPHENYL)-N-{3-[1-(4-OXO-4-PHENYLBUTYL)-4-

30

PIPERIDINYL]PHENYL}ACETAMIDE: ¹H NMR (400 MHz, CDCl₃) δ 7.98 (d, 2H, J=7.6 Hz), 7.56 (t, 1H, J=7.62 Hz), 7.46 (t, 2H, J=7.6 Hz), 7.40 (s, 1H), 7.37-7.26 (m, 2H), 7.19 (t, 1H, J=7.8 Hz), 6.94-6.86 (m, 3H), 3.81 (s, 3H), 3.71

(s, 3H), 3.12-3.03 (m, 4H), 2.57-2.44 (m, 3H), 2.16-1.77 (m, 8H); ESMS m/e : 471.3 (M + H)⁺.

Example 77

5 **N- (3-{1-[4-(2,4-DIMETHOXYPHENYL)-4-OXOBUTYL]-4-PIPERIDINYLPHENYL)ACETAMIDE:** ¹H NMR (400 MHz, CDCl₃) δ 7.82 (d, 1H, J=8.8 Hz), 7.54 (d, 1H, J=7.6 Hz), 7.33 (s, 1H), 7.22 (t, 1H, J=7.6 Hz), 6.93 (d, 1H, J=7.6 Hz), 6.53 (d, 1H, J=8.8 Hz), 6.46 (s, 1H), 3.90 (s, 3H), 3.86 (s, 3H), 3.48-3.27 (m, 2H), 3.05 (t, 2H, J=6.8 Hz), 2.90-2.68 (m, 2H), 2.65-2.38 (m, 3H), 2.25 (s, 3H), 2.18-1.80 (m, 6H); ESMS m/e : 425.3 (M + H)⁺.

Example 78

15 **N- (3-{1-[4-(2,4-DIMETHOXYPHENYL)-4-OXOBUTYL]-4-PIPERIDINYLPHENYL}-2-METHYLPROPANAMIDE:** ¹H NMR (400 MHz, CDCl₃) δ 7.98 (d, 1H, J=8.6 Hz), 7.41-7.37 (m, 2H), 7.24 (t, 1H, J=7.8 Hz), 6.96 (d, 1H, J=7.8 Hz), 6.54 (d, 1H, J=8.6 Hz), 6.46 (s, 1H), 3.89 (s, 3H), 3.86 (s, 3H), 3.11-3.08 (m, 2H), 2.98 (t, 2H, J=7.2 Hz), 2.53-2.46 (m, 4H), 2.13-1.79 (m, 8H), 1.25 (d, 6H, J=6.8 Hz); ESMS m/e : 453.3 (M + H)⁺.

Example 79

25 **N- (3-{1-[4-(2,4-DIMETHOXYPHENYL)-4-OXOBUTYL]-4-PIPERIDINYLPHENYL}-2-PHENYLACETAMIDE:** ¹H NMR (400 MHz, CDCl₃) δ 7.85 (m, 12H), 3.89 (s, 3H), 3.86 (s, 3H), 3.74 (s, 2H), 3.22-2.90 (m, 4H), 2.64-2.40 (m, 3H), 2.25-1.70 (m, 8H); ESMS m/e : 501.3 (M + H)⁺.

30

Example 80

N- (3-{1-[4-(2,4-DIMETHOXYPHENYL)-4-OXOBUTYL]-4-PIPERIDINYLPHENYL}-2-(3-METHOXYPHENYL)ACETAMIDE: ¹H NMR

(400 MHz, CDCl₃) δ 7.82 (d, 1H, J=8.8 Hz), 7.48-7.15 (m, 5H), 6.95-6.80 (m, 3H), 6.58-6.45 (m, 2H), 3.89 (s, 3H), 3.86 (s, 3H), 3.81 (s, 3H), 3.72 (s, 2H), 3.25-2.95 (m, 4H), 2.65-2.40 (m, 3H), 2.30-1.95 (m, 4H), 1.93-1.72 (m, 4H); ESMS m/e : 531.3 (M + H)⁺.

5

Example 81

N-(3-{1-[4-OXO-4-(4-PHOXYPHENYL)BUTYL]-4-

PIPERIDINYL}PHENYL)ACETAMIDE: ¹H NMR (400 MHz, CDCl₃) δ 8.15-6.75 (m, 13H), 3.30-2.80 (m, 4H), 2.75-2.10 (m, 5H), 2.03 (s, 3H), 2.00-1.60 (m, 6H); ESMS m/e : 457.3 (M + H)⁺.

10

Example 82

2-METHYL-N-(3-{1-[4-OXO-4-(4-PHOXYPHENYL)BUTYL]-4-

PIPERIDINYL}PHENYL)PROPANAMIDE: ¹H NMR (400 MHz, CDCl₃) δ 7.96 (d, 2H, J=8.8 Hz), 7.43-7.15 (m, 6H), 7.10-6.93 (m, 5H), 3.42-2.95 (m, 4H), 2.80-2.45 (m, 4H), 2.20-1.80 (m, 8H), 1.14 (d, 6H, J=6.8 Hz); ESMS m/e : 485.4 (M + H)⁺.

20

Example 83

2-(3-METHOXYPHENYL)-N-(3-{1-[4-OXO-4-(4-

PHENOXYPHENYL)BUTYL]-4-PIPERIDINYL}PHENYL)ACETAMIDE: ¹H NMR (400 MHz, CDCl₃) δ 7.97 (d, 2H, J=8.8 Hz), 7.41-7.18 (m, 7H), 7.08-6.99 (m, 5H), 6.94-6.87 (m, 3H), 3.82 (s, 3H), 3.70 (s, 2H), 3.10-2.95 (m, 4H), 2.55-2.40 (m, 3H), 2.15-1.95 (m, 4H), 1.81-1.70 (m, 4H); ESMS m/e : 563.4 (M + H)⁺.

25

Example 84

N'-(3-{1-[4-(4-CHLOROPHENYL)-4-OXOBUTYL]-4-

PIPERIDINYL}PHENYL)-N,N-DIMETHYLSULFAMIDE: ¹H NMR (400 MHz, CDCl₃) δ 7.93 (d, 2H, J=8.8 Hz), 7.44 (d, 2H, J=8.8 Hz), 7.27 (s, 1H), 7.25-7.10 (m, 2H), 6.94 (d, 1H, J=7.6

Hz), 3.30-3.10 (m, 2H), 3.04 (t, 2H, J=6.8 Hz), 2.83 (s, 6H), 2.68-2.45 (m, 3H), 2.30-1.75 (m, 8H); ESMS m/e : 464.3 (M + H)⁺.

5 **Example 85**

N-(3-{1-[4-OXO-4-(2-THIENYL)BUTYL]-4-

PIPERIDINYL}PHENYL)ACETAMIDE: ¹H NMR (400 MHz, CDCl₃) δ 7.90-6.78 (m, 7H), 3.22-2.88 (m, 4H), 2.69-2.25 (m, 5H), 2.02 (s, 3H), 2.00-1.64 (m, 6H); ESMS m/e : 371.2 (M + H)⁺.

10

Example 86

N-(3-{1-[4-(4-ISOPROPYLPHENYL)-4-OXOBUTYL]-4-

PIPERIDINYL}PHENYL)ACETAMIDE: ¹H NMR (400 MHz, CDCl₃) δ 8.00-6.78 (m, 8H), 3.15-2.98 (m, 4H), 2.77-2.15 (m, 4H), 2.03 (s, 3H), 2.00-1.62 (m, 8H), 0.927 (d, 6H, J=6.0 Hz); ESMS m/e : 407.3 (M + H)⁺.

15

Example 87

N-(3-{1-[4-(4-METHYLPHENYL)-4-OXOBUTYL]-4-

PIPERIDINYL}PHENYL)ACETAMIDE: ¹H NMR (400 MHz, CDCl₃) δ 7.90-6.80 (m, 8H), 3.10-2.45 (m, 7H), 2.32 (s, 3H), 2.02 (s, 3H), 2.01-1.68 (m, 8H); ESMS m/e : 379.3 (M + H)⁺.

20

Example 88

N-(3-{1-[4-(4-BROMOPHENYL)-4-OXOBUTYL]-4-

PIPERIDINYL}PHENYL)ACETAMIDE: ¹H NMR (400 MHz, CDCl₃) δ 7.90-6.80 (m, 8H), 3.30-3.05 (m, 4H), 2.70-2.45 (m, 3H), 2.05 (s, 3H), 1.98-1.65 (m, 8H); ESMS m/e : 444.0 (M + H)⁺.

25

EXAMPLE 89

N- (3-{1-[4-(3,4-DIMETHYLPHENYL)-4-OXOBUTYL]-4-

PIPERIDINYL}PHENYL)-2-PROPANESULFONAMIDE: ^1H NMR (400 MHz, CDCl_3) δ 7.75 (s, 1H), 7.71 (d, 1H, $J=7.6$ Hz), 7.27-7.00 (m, 5H), 3.32-3.24 (m, 3H), 3.10-3.02 (m, 2H), 5 2.78-2.50 (m, 3H), 2.32 (s, 6H), 2.19-1.84 (m, 8H), 1.39 (d, 6H, $J=6.8$ Hz); ESMS m/e : 457.4 ($M + H$)⁺.

Example 90

N- (3-{1-[4-OXO-4-(4-PHOXYPHENYL)BUTYL]-4-

10 PIPERIDINYL}PHENYL)-2-PROPANESULFONAMIDE: ^1H NMR (400 MHz, CDCl_3) δ 7.97 (d, 2H, $J=7.6$ Hz), 7.44 (t, 2H, $J=7.6$ Hz), 7.27-7.00 (m, 9H), 3.35-2.96 (m, 5H), 2.69-2.45 (m, 3H), 2.14-1.79 (m, 8H), 1.39 (d, 6H, $J=6.8$ Hz); ESMS m/e : 521.4 ($M + H$)⁺.

15

Example 91

N- (3-{1-[3-(4-CHLOROPHENYL)-3-METHOXYPROPYL]-4-

PIPERIDINYL}PHENYL)-2-METHYLPROPANAMIDE

A mixture of 3-methoxy-3-(p-chlorophenyl)-1-20 chloropropane (27.4 mg, 0.125 mmol), 2-methyl-N-[3-(4-piperidinyl)phenyl]propanamide (28.3 mg, 0.125 mmol), diisopropylethylamine (0.50 mL) and catalytic amount of tetrabutylammonium iodide in dioxane (2.0 mL) was stirred at 90 °C for 72 hrs. The reaction mixture was concentrated to a small volume and chromatographed using preparative TLC plates [2.5% of NH_3 (2.0 M in methanol) 25 in CHCl_3] gave N-(3-{1-[3-(4-chlorophenyl)-3-methoxypropyl]-4-piperidinyl}phenyl)-2-methylpropanamide (39.5 mg, 73.8% yield) as a thick oil: ^1H NMR δ 7.48 (s, 1H), 7.34-7.3 (m, 2H), 7.25 (m, 4H), 6.96 (d, 1H, $J=7.4$ Hz), 4.20 (apparent dd, 1H, $J=5.9, 7.6$ Hz), 3.2 (s, 3H), 3.04 (d, 1H, $J=10.1$ Hz), 2.99 (d, 1H, $J=10.1$ Hz), 2.49

(h, 4H, J=6.6 Hz), 2.20-2.10 (m, 4H), 1.82 (m, 4H), 1.25 (d, 6H, J=7.1 Hz); ESMS m/e: 429.4 (M + H)⁺.

Example 92

5 N- (3-{1-[6-(1,3-DIOXO-1,3-DIHYDRO-2H-ISOINDOL-2-YL)HEXYL]-4-PIPERIDINYL}PHENYL)-2-METHYLPROPANAMIDE: The synthetic method is the same as described for 2-[6-(4-phenyl-1-piperidinyl)hexyl]-1H-isoindole-1,3(2H)-dione.
10 N- (3-{1-[6-(1,3-dioxo-1,3-dihydro-2H-isoindol-2-yl)hexyl]-4-piperidinyl}phenyl)-2-methylpropanamide: 506 mg (56% yield); ¹H NMR (400 MHz, CDCl₃) δ 7.86-7.80 (m, 2H), 7.73-7.68 (m, 2H), 7.44 (s, 1H), 7.37 (d, 1H, J=8.3 Hz), 7.22 (t, 1H, J=7.7 Hz), 6.96 (d, 1H, J=7.7 Hz), 3.69 (t, 2H, J=7.2 Hz), 3.01 (apparent d, 2H, J=11.3 Hz), 2.58-2.40 (m, 2H), 2.33 (m, 2H) 1.98 (dt, 2H, J=3.2, 11.3 Hz), 1.84-1.64 (m, 4H), 1.51 (q, 2H, J=7.1 Hz), 1.43-1.30 (m, 6H), 1.24 (d, 6H, J=6.8 Hz); ESMS m/e: 476.4 (M + H)⁺.

20 **Example 93**

N-{3-[1-(3-METHOXY-3-PHENYLPROPYL)-4-PIPERIDINYL]PHENYL}-2-METHYLPROPANAMIDE

25 A mixture of 3-methoxy-3-phenyl-1-chloropropane (23.1 mg, 0.126 mmol), 2-methyl-N-[3-(4-piperidinyl)phenyl]propanamide (28.3 mg, 0.126 mmol), diisopropylethylamine (0.50 mL) and catalytic amount of tetrabutylammonium iodide in dioxane (2.0 mL) was
30 stirred at 90 °C for 72 hrs. Chromatography using silica preparative TLC plates [2.5% of NH₃ (2.0 M in methanol) in CHCl₃] gave N-{3-[1-(3-methoxy-3-phenylpropyl)-4-piperidinyl]phenyl}-2-methylpropanamide (45.4 mg, 91.2%

yield) as a thick oil: ^1H NMR (400 MHz, CDCl_3) δ 7.45 (s, 1 H), 7.34-7.25 (m, 5H), 7.25 (m, 2H), 6.96 (d, 1H, $J=7.4$ Hz), 4.20 (apparent dd, 1H, $J=5.9, 7.6$ Hz), 3.2 (s, 3H), 3.04 (d, 1H, $J=10.1$ Hz), 2.99 (d, 1H, $J=10.1$ Hz), 2.49 (apparent sept, partially hidden, 4H, $J=6.6$ Hz), 2.3-2.1 (m, 4H), 1.82 (m, 4H), 1.25 (d, 6H, $J=7.1$ Hz); ESMS m/e: 395.4 ($M + H$) $^+$.

Example 94

N-(3-{1-[4-(1,3-DIOXO-1,3-DIHYDRO-2H-ISOINDOL-2-YL)BUTYL]-4-PIPERIDINYL}PHENYL)-2-METHYLPROPANAMIDE: The synthetic method is the same as described for 2-[6-(4-phenyl-1-piperidinyl)hexyl]-1H-isoindole-1,3(2H)-dione. N-(3-{1-[4-(1,3-dioxo-1,3-dihydro-2H-isoindol-2-yl)butyl]-4-piperidinyl}phenyl)-2-methylpropanamide: 664 mg (74% yield); ^1H NMR (400 MHz, CDCl_3) δ 7.87-7.78 (m, 2H), 7.76-7.64 (m, 2H), 7.47 (s, 1H), 7.39 (d, 1H, $J=7.6$ Hz), 7.21 (t, 1H, $J=8.1$ Hz), 6.94 (d, 1H, $J=7.6$ Hz), 3.72 (t, 2H, $J=6.8$ Hz), 3.37-3.22 (m, 2H), 3.0 (apparent d, 2H, $J=10.7$ Hz), 2.75 (q, 2H, $J=7.0$ Hz), 2.64-2.33 (m, 4H), 1.99 (dt, 2H, $J=2.6, 11.7$ Hz), 1.86-1.65 (m, 2H), 1.63-1.50 (m, 2H), 1.23 and 1.21 (two d, 6H, $J=5.5$ Hz); ESMS m/e: 448.4 ($M + H$) $^+$; Anal. Calc. for $C_{27}\text{H}_{34}\text{N}_3\text{ClO}_3 + 0.4\text{H}_2\text{O}$: C, 66.02; H, 7.14; N, 8.55. Found: C, 66.07; H, 6.78; N, 8.65.

Example 95

N-(3-{1-[4-(1,3-DIOXO-1,3-DIHYDRO-2H-ISOINDOL-2-YL)BUTYL]-4-PIPERIDINYL}PHENYL)-2-METHYLPROPANAMIDE: The synthetic method is the same as described for 2-[6-(4-phenyl-1-piperidinyl)hexyl]-1H-isoindole-1,3(2H)-dione. N-(3-{1-[5-(1,3-dioxo-1,3-dihydro-2H-isoindol-2-yl)pentyl]-4-piperidinyl}phenyl)-2-methylpropanamide:

614 mg (64% yield); ^1H NMR (400 MHz, CDCl_3) δ 7.87-7.8 (m, 2H), 7.76-7.68 (m, 2H), 7.48 (s, 1H), 7.41 (d, 1H, $J=7.6$ Hz), 7.21 (t, 1H, $J=7.6$ Hz), 6.95 (d, 1H, $J=7.6$ Hz), 3.69 (t, 2H, $J=7.2$ Hz), 3.39-3.28 (m, 2H), 3.02 (apparent d, 2H, $J=11.6$ Hz), 2.78 (q, 2H, $J=7.2$ Hz), 2.64-2.52 (m, 1H), 2.52-2.40 (m, 1H), 2.40-2.31 (m, 2H), 2.01 (dt, 2H, $J=3.7$, 11.1 Hz), 1.85-1.64 (m, 2H), 1.58 (q, 2H, $J=7.6$ Hz), 1.45-1.32 (m, 2H), 1.23 (d, 6H, $J=6.9$ Hz); ESMS m/e: 462.4 ($M + H$) $^+$; Anal. Calc. for $\text{C}_{28}\text{H}_{36}\text{N}_3\text{ClO}_3$: C, 67.52; H, 7.29; N, 8.44. Found: C, 67.04; H, 7.06; N, 8.38.

Example 96

2-METHYL-N-[3-[1-(4-PHENYLBUTYL)-4-
15 PIPERIDINYL]PHENYL]PROPANAMIDE

A mixture of 2-methyl-N-[3-(4-piperidinyl)phenyl]propanamide (28.3 mg, 0.100 mmol), 4-phenyl-1-chlorobutane (21.1 mg, 0.125 mmol), diisopropylethylamine (0.50 mL), catalytic amount of tetrabutylammonium iodide and dioxane (2.0 mL) was heated at reflux temperature for 3 days. The reaction mixture was concentrated and chromatographed using preparative TLC plates [2.5% of NH_3 (2.0 M in methanol) in CHCl_3] afforded the product, 2-methyl-N-[3-[1-(4-phenylbutyl)-4-piperidinyl]phenyl]propanamide (9.50 mg, 25.1% yield) as a thick oil: ^1H NMR δ 7.37 (s, 1H), 7.29 (apparent d, 1H, $J=7.9$ Hz), 7.18 (m, 3H), 7.11 (m, 3H), 6.90 (apparent d, 1H, $J=7.9$ Hz), 3.02 (d, 2H, $J=6.8$ Hz), 2.41 (m, 4H, partially hidden), 2.01 (m, 2H), 1.78 (m, 4H), 1.57 (m, 4H), 1.18 (d, 6H, $J=7.7$ Hz); ESMS m/e: 379.4 ($M + H$) $^+$.

Example 97

N- (3-{1-[3-(1,3-DIOXO-1,3-DIHYDRO-2H-ISOINDOL-2-YL)PROPYL]-4-PIPERIDINYL}PHENYL)-2-METHYLPROPANAMIDE:

The synthetic method is the same as described for 2-[6-(4-phenyl-1-piperidinyl)hexyl]-1H-isoindole-1,3 (2H)-dione.

5

N- (3-{1-[3-(1,3-dioxo-1,3-dihydro-2H-isoindol-2-yl)propyl]-4-piperidinyl}phenyl)-2-methylpropanamide:

810 mg (93% yield); ^1H NMR (400 MHz, CDCl_3) δ 7.87-7.82 (m, 2H), 7.73-7.68 (m, 2H), 7.57 (s, 1H), 7.36 (d, 1H, $J=8.5$ Hz), 7.18 (t, 1H, $J=7.7$ Hz), 6.79 (d, 1H, $J=7.1$ Hz), 3.78 (t, 2H, $J=6.8$ Hz), 3.06 (quintet, 2H, $J=6$ Hz), 2.95 (apparent d, 2H, $J=12.2$ Hz), 2.58-2.31 (m, 4H), 1.96-1.83 (m, 2H), 1.70 (apparent d, 2H, $J=12.1$ Hz), 1.52 (dt, 2H, $J=3.5$, 12.5 Hz), 1.03 (d, 6H, $J=6.5$ Hz);
10
15
ESMS m/e: 434.4 ($M + H$)⁺.

Example 98

N- (3-{1-[(3S)-3-HYDROXY-3-PHENYLPROPYL]-4-PIPERIDINYL}PHENYL)-2-METHYLPROPANAMIDE

20 A mixture of (S)-(-)-3-chloro-1-phenyl-1-propanol (0.426 g, 2.50 mmol, 99%ee), 2-methyl-N-[3-(4-piperidinyl)phenyl]propanamide (0.565 g, 2.00 mmol), diisopropylethylamine (1.29 g, 10.0 mmol), dioxane (5.0 mL) and catalytic amount of tetrabutylammonium iodide
25 was stirred at 90 °C for 72 hrs. Chromatography using silica preparative TLC plates [2.5% of NH_3 (2.0 M in methanol) in CHCl_3] gave the desired product (306 mg, 39.3 % yield) as a thick oil: ^1H NMR (400 MHz, CDCl_3) δ 7.46 (s, 1 H), 7.42 (d, 4H, $J=8.1$ Hz), 7.35 (m, 1 H), 7.30 (d, 1 H, $J=8.0$ Hz), 7.23 (t, 1H, $J=8.1$ Hz), 7.12 (s, 1H), 6.96 (apparent dd, 1H, $J=8.0$ Hz), 5.0 (apparent dd, 1H, $J=4.4$, 8.3 Hz), 3.18 (apparent dd, 2H, $J=2.5$, 12.5 Hz), 2.74 (m, 2 H), 2.50 (m, 2H), 2.3-2.1 (m, 6H),
30

1.8 (m, 2H), 1.25 (d, 6H, J=7.1 Hz); ESMS m/e: 389.2 (M + H)⁺.

Example 99

5 **N-(3-[1-[3-METHOXY-3-(4-METHYLPHENYL)PROPYL]-4-PIPERIDINYL]PHENYL)-2-METHYLPROPANAMIDE**

A mixture of 3-methoxy-3-(p-tolyl)-1-chloropropane (24.9 mg, 0.126 mmol), 2-methyl-N-[3-(4-piperidinyl)phenyl]propanamide (28.3 mg, 0.126 mmol), diisopropylethylamine (0.50 mL) and catalytic amount of tetrabutylammonium iodide in dioxane (2.0 mL) was stirred at 90 °C for 72 hrs. Chromatography using silica preparative TLC plates [2.5% of NH₃ (2.0 M in methanol) in CHCl₃] gave the desired product (10.9 mg, 21.2 % yield) as a thick oil: ¹H NMR (400 MHz, CDCl₃) δ 7.44 (s, 1 H), 7.38 (m, 1H), 7.3-7.1 (m, 5 H), 6.96 (d, 1H, J=7.4 Hz), 4.18 (apparent dd, 1H, J=5.6, 7.9 Hz), 3.24 (d, 1H, J=8.2 Hz), 3.2 (s, 3H), 3.11 (m, 2H, J=10.1Hz), 2.49 (m, 4H), 2.35 (s, 3H), 2.3-2.1(m, 3H), 1.92 (d, 4H), 1.25 (d, 6H, J=7.1 Hz); ESMS m/e: 409.4 (M + H)⁺.

Example 100

25 **N-[3-[1-(3-ISOPROPOXY-3-PHENYLPROPYL)-4-PIPERIDINYL]PHENYL]-2-METHYLPROPANAMIDE**

A mixture of 3-isopropyl-3'-phenyl-1-chloropropane (26.6 mg, 0.126 mmol), 2-methyl-N-[3-(4-piperidinyl)phenyl]propanamide (28.3 mg, 0.126 mmol), diisopropylethylamine (0.50 mL) and catalytic amount of tetrabutylammonium iodide in dioxane (2.0 mL) was stirred at 90 °C for 72 hrs. Chromatography using silica preparative TLC plates [2.5% of NH₃ (2.0 M in methanol) in CHCl₃] gave the desired product (14.1 mg, 26.5% yield) as a thick oil: ¹H NMR (400 MHz, CDCl₃) δ 7.46 (s, 1H),

7.43-7.37 (m, 2H), 7.33 (m, 3H), 7.23 (m, 2H), 6.95 (d, 1H, J=8.4 Hz), 4.46 (apparent dd, 1H, J=5.0, 8.3 Hz), 3.49 (apparent sept, 1H, J=7.1 Hz), 3.10 (s, 2H), 2.70 (m, 2H), 2.52 (apparent sept, partially hidden, 4H, J=6.6 Hz), 2.30-2.10 (m, 2H), 1.90-1.80 (d, 4H), 1.25 (d, 6H, J=7.1 Hz), 1.15 (d, 3H, J=6.4 Hz), 1.08 (d, 3H, J=6.4 Hz); ESMS *m/e*: 423.4 (M + H)⁺.

Example 101

10 **N-(3-{1-[4,4-BIS(4-FLUOROPHENYL)BUTYL]-4-PIPERIDINYLPHENYL}-2-METHYLPROPANAMIDE**

A mixture of 4,4-bis(4-fluoro-phenyl)-1-chloro-butane (39.0 mg, 0.126 mmol), 2-methyl-N-[3-(4-piperidinyl)phenyl]propanamide (28.3 mg, 0.126 mmol), diisopropylethylamine (0.50 mL) and catalytic amount of tetrabutylammonium iodide in dioxane (2.0 mL) was stirred at 90 °C for 72 hrs. Chromatography using silica preparative TLC plates [2.5% of NH₃ (2.0 M in methanol) in CHCl₃] gave the desired product (15.9 mg, 25.2 % yield) as a thick oil: ¹H NMR (400 MHz, CDCl₃) δ 8.02 (s, 1H), 7.41 (s, 1H), 7.3-7.15 (m, 4H), 7.10 (m, 3H), 6.89 (apparent t, 5H), 3.81 (t, 1H, J=7.8 Hz), 3.30 (s, 1H), 2.91 (d, 1H, J=12.5 Hz), 2.80 (m, 1H), 2.40 (m, 2H), 2.31 (t, 1H, J=8.0 Hz), 1.93 (apparent q, 3H, J=8.0 Hz), 1.72 (m, 3H), 1.40 (m, 2H), 1.20 (m, 2H), 1.15 (d, 6H, J=8.1 Hz); ESMS *m/e*: 491.4 (M + H)⁺

EXAMPLE 102

30 **N-{3-[1-(3-METHOXYBENZYL)-4-PIPERIDINYLPHENYL]-2-METHYLPROPANAMIDE**

A mixture of 2-methyl-N-[3-(4-piperidinyl)phenyl]propanamide (28.3 mg, 0.100 mmol), 3-methoxybenzyl chloride (19.6 mg, 0.125 mmol),

diisopropylethylamine (0.50 mL), catalytic amount of tetrabutylammonium iodide and dioxane (2.0 mL). Chromatography using silica preparative TLC plates [2.5% of NH₃ (2.0 M in methanol) in CHCl₃] afforded the desired product (10.2 mg, 27.9% yield) as a yellow solid: ¹H NMR (400 MHz, CDCl₃) δ 7.46 (s, 1H), 7.35 (apparent d, 1H, J=8.3 Hz), 7.27-7.21 (m, 2H), 6.95 (apparent t, 3H, J=6.9 Hz), 6.82 (apparent dd, 1H, J=2.4, 8.3 Hz), 3.84 (m, 3H), 3.56 (s, 2H), 3.05 (d, 2H, J=10.5 Hz), 2.51 (apparent sept, partially hidden, 4H, J=7.2 Hz), 2.13 (apparent t, 2H, J=9.7 Hz), 1.88 (m, 2H), 1.25 (d, 6H, J=6.7 Hz); ESMS m/e: 367.3 (M + H)⁺.

Example 103

N- (3-[1-[3,5-BIS(TRIFLUOROMETHYL)BENZYL]-4-PIPERIDINYLPHENYL]-2-METHYLPROPANAMIDE
A mixture of 2-methyl-N-[3-(4-piperidinyl)phenyl]propanamide (28.3 mg, 0.100 mmol), 3,5-bis(trifluoromethyl)benzyl bromide (38.4 mg, 0.125 mmol), diisopropylethylamine (0.50 mL), catalytic amount of tetrabutylammonium iodide and dioxane (2.0 mL). Chromatography using silica preparative TLC plates [2.5% of NH₃ (2.0 M in methanol) in CHCl₃] gave the desired product (12.2 mg, 25.8% yield) as a thick oil: ¹H NMR (400 MHz, CDCl₃) δ 7.83 (s, 2H), 7.77 (s, 1H), 7.53 (s, 1H), 7.30-7.21 (m, 2H), 7.16 (s, 1H), 6.98 (apparent d, 1H, J=7.6 Hz), 3.62 (s, 2H), 2.94 (d, 2H, J=9.4 Hz), 2.51 (apparent sept, partially hidden, 2H, J=6.6 Hz), 2.14 (m, 2H), 1.82 (m, 4H), 1.25 (d, 6H, J=6.6 Hz); ESMS m/e: 473.2 (M + H)⁺.

Example 104

N- (3-{1- [(3R)-3-(3,4-DIMETHOXYPHENOXY)-3-PHENYLPROPYL]-
4-PIPERIDINYL}PHENYL)-2-METHYLPROPANAMIDE

Method A

5 4-{[(1R)-3-chloro-1-phenylpropyl]oxy}-1,2-dimethoxybenzene:

A mixture of 3,4-dimethoxyphenol (4.07 g, 26.4 mmol), (S)-(-)-3-chloro-phenyl-1-propanol (4.50 g, 26.4 mmol, 99%ee, Aldrich Chemical Co.), triphenylphosphine (6.92 g, 26.4 mmol) and diethyl azodicarboxylate (4.59 g, 26.4 mmol) in THF (110 mL) was stirred at room temperature for 24 h. The reaction mixture was concentrated *in vacuo*.

15 At this point, the residue can either be washed with pentane (x3) and the combined pentane extracts were concentrated and chromatographed (silica with hexanes-EtOAc 8:1 as the eluent) to give the desired product (as described as a general procedure by: Srebnik, M.; Ramachandran, P.V.; Brown, H.C. *J. Org. Chem.* **1988**, *53*, 2916-2920). This procedure was performed on a smaller scale reaction and only a 40% yield of the product was realized.

20 Alternatively, on a larger scale (26.4 mmol), the crude product was triturated with a small amount of dichloromethane and the precipitated triphenylphosphine oxide was filtered. The filtrate was concentrated and the crude product was chromatographed to give the desired product as a thick yellow oil (7.30 g, 88.9% yield): ¹H NMR (400 MHz, CDCl₃) δ 7.39-7.32 (m, 4H), 7.20 (m, 1H), 6.64 (d, 1H, J=8.7 Hz), 6.51 (d, 1H, J=2.7 Hz), 6.30 (dd, 1H, J=2.7, 8.7 Hz), 5.27 (apparent dd, 1H, J=4.5, 8.7 Hz), 3.79 (s, 3H), 3.77 (s, 3H), 3.61 (m,

1H), 2.45 (m, 1 H), 2.20 (m, 1H), 1.80 (s, 1H); ESMS
m/e: 307.11 (M+H)⁺.

N- (3-{1- [(3R)-3-(3,4-DIMETHOXYPHOXY)-3-PHENYLPROPYL]-
5 4-PIPERIDINYL}PHENYL)-2-METHYLPROPANAMIDE: A mixture of
potassium carbonate (321 mg, 2.32 mmol), sodium iodide
(522 mg, 3.48 mmol), 2-methyl-N-[3-(4-
10 piperidinyl)phenyl]propanamide (570 mg, 2.32 mmol) and
4-{[(1R)-3-chloro-1-phenylpropyl]oxy}-1,2-
dimethoxybenzene (712 mg, 2.32 mmol) in DMF (5.0 mL) was
stirred at 100 °C for 3 hrs, at which time TLC indicated
that the reaction was complete. The reaction mixture
was poured into water (50 mL) and the aqueous layer was
extracted with methylene chloride (3x30 mL). The
15 combined organic extracts were washed with brine (30
mL), dried over MgSO₄ and concentrated under reduced
pressure. The crude product was purified by Prep-TLC
plates [2.5% of NH₃ (2.0 M in methanol) in CHCl₃] to
afford the product (970 mg, 90.1%) as a thick oil.

20

Method B

Into a 25-mL RB-flask was added triphenylphosphine (9.80
mg, 0.0375 mmol), diethyl azodicarboxylate (5.22 mg,
0.0300 mmol), N-(3-{1-[(3S)-3-hydroxy-3-phenylpropyl]-4-
25 piperidinyl}phenyl)-2-methylpropanamide (9.53 mg, 0.0250
mmol), 3,4-dimethoxyphenol (7.70 mg, 0.050 mmol) and THF
(1.0 mL) at room temperature. The reaction mixture was
stirred at room temperature overnight (16 hrs). The
solvent was removed under reduced pressure and the
30 residue was purified by preparative TLC plates [2.5% of
NH₃ (2.0 M in methanol) in CHCl₃] to afford the desired
product (4.4 mg, 34.1 % yield) as a thick oil: ¹H NMR
(400 MHz, CDCl₃) δ 7.46 (s, 1 H), 7.40-7.30 (m, 4H),

7.25 (m, 3H), 6.97 (d, 1H, J=7.8 Hz), 6.64 (d, 1H, J=9.1 Hz), 6.51 (d, 1H, J=2.6 Hz), 6.29 (d, 1H, J=2.6, 9.1 Hz), 5.20 (apparent dd, 1H, J=4.4, 8.5 Hz), 3.80 (s, 3H), 3.77 (s, 3H), 3.23 (m, 2H), 2.77 (m, 2 H), 2.5 (m, 2H), 2.3-2.1(m, 6H), 1.80 (m, 2H), 1.25 (d, 6H, J=7.9 Hz); ESMS *m/e*: 517.4 (M + H)⁺.

Example 105

2-METHYL-N-(3-{1-[(3S)-3-PHOXY-3-PHENYLPROPYL]-4-PIPERIDINYLPHENYL)PROPANAMIDE

A mixture of N-(3-{1-[(3R)-3-hydroxy-3-phenylpropyl]-4-piperidinyl}phenyl)-2-methylpropanamide (9.53 mg, 0.0250 mmol), phenol (4.70 mg, 0.050 mmol), triphenylphosphine (9.80 mg, 0.0375 mmol) and diethyl azodicarboxylate (5.22 mg, 0.0300 mmol) in THF (1.0 mL) was stirred at room temperature for 3 days. Chromatography using silica preparative TLC plates [2.5% of NH₃ (2.0 M in methanol) in CHCl₃] gave the desired product (2.7 mg, 23.6 % yield) as a thick oil: ¹H NMR δ 7.46 (s, 2H), 7.40-7.30 (m, 4H), 7.25 (m, 3 H), 7.20 (m, 2H), 6.97 (apparent d, 1H, J=7.4 Hz), 6.89 (apparent tt, 1H, J=0.8, 7.6 Hz), 6.84 (apparent dt, 1H, J=0.8, 8.0 Hz), 5.20 (apparent dd, 1H, J=4.4, 8.5 Hz), 3.35 (m, 2H), 2.91 (m, 2H), 2.60 (m, 2H), 2.30-2.10 (m, 6H), 1.90 (m, 2H), 1.25 (d, 6H, J=7.9 Hz); ESMS *m/e*: 457.4 (M + H)⁺;

Example 106

N-(3-{1-[(3S)-3-(4-METHOXYPHENOXY)-3-PHENYLPROPYL]-4-PIPERIDINYLPHENYL)-2-METHYLPROPANAMIDE

A mixture of N-(3-{1-[(3R)-3-hydroxy-3-phenylpropyl]-4-piperidinyl}phenyl)-2-methylpropanamide (9.53 mg, 0.0250 mmol), 4-methoxyphenol (6.20 mg, 0.050 mmol), triphenylphosphine (9.80 mg, 0.0375 mmol) and diethyl

azodicarboxylate (5.2 mg, 0.0300 mmol) in THF (1.0 mL) was stirred at room temperature for 3 days. Chromatography using silica preparative TLC plates [2.5% of NH₃ (2.0 M in methanol) in CHCl₃] gave the desired product (4.6 mg, 37.9 % yield) as a thick oil. ¹H NMR (400 MHz, CDCl₃) δ 7.38-7.14 (m, 8H), 6.90 (apparent d, 1H, J=7.7 Hz), 6.72-6.46 (m, 4H), 5.09 (apparent dd, 1H, J=4.8, 8.1 Hz), 3.64 (s, 3H), 3.18 (m, 2H), 2.73 (m, 2H), 2.50 (m, 2H), 2.37-1.72 (m, 8H), 1.25 (d, 6H, J=7.4 Hz); ESMS m/e: 487.4 (M + H)⁺.

Example 107

N-(3-{1-[(3S)-3-(3-CHLOROPHOXY)-3-PHENYLPROPYL]-4-PIPERIDINYL}PHENYL)-2-METHYLPROPANAMIDE A mixture of N-(3-{1-[(3R)-3-hydroxy-3-phenylpropyl]-4-piperidinyl}phenyl)-2-methylpropanamide (9.53 mg, 0.0250 mmol), 3-chlorophenol (6.40 mg, 0.050 mmol), triphenylphosphine (9.80 mg, 0.0375 mmol) and diethyl azodicarboxylate (5.22 mg, 0.0300 mmol) in THF (1.0 mL) was stirred at room temperature for 3 days.

Chromatography using silica preparative TLC plates [2.5% of NH₃ (2.0 M in methanol) in CHCl₃] gave the desired product (4.9 mg, 40.0 % yield) as a thick oil: ¹H NMR (400 MHz, CDCl₃) δ 7.39 (s, 1H), 7.35-7.10 (m, 7H), 7.02 (t, 1H, J=8.0 Hz), 6.90 (d, 1H, J=7.6 Hz), 6.84-6.75 (m, 2H), 6.65 (m, 1H), 5.09 (apparent dd, 1H, J=4.99, 8.1 Hz), 3.10 (m, 2H), 2.60 (m, 2H), 2.50 (m, 2H), 2.30-1.70 (m, 8H), 1.18 (d, 6H, J=6.8 Hz); ESMS m/e: 491.4 (M + H)⁺.

30

Example 108

N-(3-{1-[(3S)-3-(4-CHLOROPHOXY)-3-PHENYLPROPYL]-4-PIPERIDINYL}PHENYL)-2-METHYLPROPANAMIDE

A mixture of N-(3-{1-[(3R)-3-hydroxy-3-phenylpropyl]-4-piperidinyl}phenyl)-2-methylpropanamide (9.53 mg, 0.0250 mmol), 4-chlorophenol (6.40 mg, 0.050 mmol), triphenylphosphine (9.80 mg, 0.0375 mmol) and diethyl azodicarboxylate (5.22 mg, 0.0300 mmol) in THF (1.0 mL) was stirred at room temperature for 3 days.

Chromatography using silica preparative TLC plates [2.5% of NH₃ (2.0 M in methanol) in CHCl₃] gave the desired product (3.3 mg, 26.9 % yield) as a thick oil: ¹H NMR δ 7.36 (s, 1H), 7.35-7.22 (m, 7H), 7.12 (m, 2H), 6.97 (apparent d, 1H, J=7.2 Hz), 6.77 (m, 2H), 5.23 (m, 1H), 3.18 (m, 2H), 2.70 (m, 2H), 2.50 (m, 2H), 2.40-1.80 (m, 8H), 1.25 (d, 6H, J=6.8 Hz); ESMS m/e: 491.4 (M + H)⁺.

15 **Example 109**

2-METHYL-N-[3-(1-{(3S)-3-PHENYL-3-[4-(TRIFLUOROMETHYL)PHENOXY]PROPYL}-4-PIPERIDINYL)PHENYL]PROPANAMIDE

A mixture of N-(3-{1-[(3R)-3-hydroxy-3-phenylpropyl]-4-piperidinyl}phenyl)-2-methylpropanamide (9.53 mg, 0.0250 mmol), 4-trifluoromethylphenol (8.100 mg, 0.050 mmol), triphenylphosphine (9.8 mg, 0.0375 mmol) and diethyl azodicarboxylate (5.22 mg, 0.0300 mmol) in THF (1.0 mL) was stirred at room temperature for 3 days.

25 Chromatography using silica preparative TLC plates [2.5% of NH₃ (2.0 M in methanol) in CHCl₃] gave the desired product (5.10 mg, 38.9 % yield) as a thick oil: ¹H NMR δ 8.06 (s, 1H), 7.49 (s, 1H), 7.44 (apparent d, 2H, J=.6 Hz), 7.38-7.30 (m, 4H), 7.30-7.20 (m, 3H), 6.96 (apparent d, 1H, J=7.6 Hz), 6.91 (apparent d, 2H, J=8.6 Hz), 5.34 (m, 1H), 3.19 (m, 2H), 2.72 (m, 2H), 2.53 (m, 2H), 2.40-1.80 (m, 8H), 1.25 (d, 6H, J=6.8 Hz); ESMS m/e: 525.4 (M + H)⁺.

Example 110

N- (3-{1-[(3R)-3-(2,5-DIFLUOROPHOXY)-3-PHENYLPROPYL]-4-PIPERIDINYL}PHENYL)-2-METHYLPROPANAMIDE

5 A mixture of N-(3-{1-[(3R)-3-hydroxy-3-phenylpropyl]-4-piperidinyl}phenyl)-2-methylpropanamide (9.53 mg, 0.0250 mmol), 2,5-difluorophenol (6.50 mg, 0.050 mmol), triphenylphosphine (9.80 mg, 0.0375 mmol) and diethyl azodicarboxylate (5.22 mg, 0.0300 mmol) in THF (1.0 mL)

10 was stirred at room temperature for 3 days.

Chromatography using silica preparative TLC plates [2.5% of NH₃ (2.0 M in methanol) in CHCl₃] gave the desired product (3.60 mg, 29.3 % yield) as a thick oil: ¹H NMR δ 7.46 (s, 1H), 7.40-7.32 (m, 4H), 7.31-7.20 (m, 2H), 7.17 (s, 1H), 7.01-6.92 (m, 2H), 6.65-6.42 (m, 2H), 5.27 (m, 1H), 3.13 (m, 2H), 2.64 (m, 2H), 2.51 (m, 2H), 2.28-1.80 (m, 8 H), 1.25 (d, 6H, J=7.1 Hz); ESMS m/e: 493.4 (M + H)⁺.

20 **Example 111**

N- (3-{1-[(3R)-3-(3,4-DICHLOROPHOXY)-3-PHENYLPROPYL]-4-PIPERIDINYL}PHENYL)-2-METHYLPROPANAMIDE

A mixture of N-(3-{1-[(3S)-3-hydroxy-3-phenylpropyl]-4-piperidinyl}phenyl)-2-methylpropanamide (9.53 mg, 0.0250 mmol), 3,4-dichlorophenol (8.20 mg, 0.050 mmol), triphenylphosphine (9.80 mg, 0.0375 mmol) and diethyl azodicarboxylate (5.22 mg, 0.0300 mmol) in THF (1.0 mL) was stirred at room temperature for 3 days.

Chromatography using silica preparative TLC plates [2.5% of NH₃ (2.0 M in methanol) in CHCl₃] gave the desired product (5.20 mg, 39.7 % yield) as a thick oil: ¹H NMR δ 7.70-7.63 (m, 2H), 7.55 (m, 1H), 7.47-7.43 (m, 3H), 7.40-7.19 (m, 3H), 7.00-6.50 (m, 2H), 6.69 (dd, 1H,

J=2.2, 8.8 Hz), 5.25 (m, 1H), 3.20 (m, 2H), 2.70 (m, 2H), 2.53 (m, 2H), 2.40-2.20 (m, 4H), 2.10-1.80 (m, 4H), 1.25 (d, 6H, J=7.1 Hz); ESMS m/e: 525.4 (M + H)⁺.

5 **Example 112**

2-METHYL-N-(3-{1-[(3R)-3-PHOXY-3-PHENYLPROPYL]-4-PIPERIDINYL}PHENYL)PROPANAMIDE

A mixture of N-(3-{1-[(3S)-3-hydroxy-3-phenylpropyl]-4-piperidinyl}phenyl)-2-methylpropanamide (9.53 mg, 0.0250 mmol), phenol (4.70 mg, 0.050 mmol), triphenylphosphine (9.80 mg, 0.0375 mmol) and diethyl azodicarboxylate (5.22 mg, 0.0300 mmol) in THF (1.0 mL) was stirred at room temperature for 3 days. Chromatography using silica preparative TLC plates [2.5% of NH₃ (2.0 M in methanol) in CHCl₃] gave the desired product (4.1 mg, 36.0 % yield) as a thick oil: ¹H NMR (400 MHz, CDCl₃) δ 7.45 (s, 1H), 7.40-7.15 (m, 10H), 6.97 (d, 1H, J=7.6 Hz), 6.88-6.82 (m, 2H), 5.26 (m, 1H), 3.18 (m, 2H), 2.75 (m, 2H), 2.53 (m, 2H), 2.40-2.10 (m, 4H), 2.10-1.80 (m, 4H), 1.25 (d, 6H, J=6.9 Hz); ESMS m/e: 457.4 (M + H)⁺.

10 **Example 113**

N-(3-{1-[(3R)-3-HYDROXY-3-PHENYLPROPYL]-4-PIPERIDINYL}PHENYL)-2-METHYLPROPANAMIDE

15

Method A

20 Into a 25-mL RB-flask was added (R)-(+)-3-chloro-1-phenyl-1-propanol (0.545 g, 3.19 mmol, 99%ee, Aldrich Chemical Co.), 2-methyl-N-[3-(4-piperidinyl)phenyl]propanamide (0.748 g, 3.04 mmol), potassium carbonate (0.420 g, 3.04 mmol) and sodium iodide (0.684 g, 4.56 mmol) and DMF (6.0 mL) at room temperature. After stirring at 100 °C for 3 hrs, the TLC

showed the reaction was complete. The reaction mixture was poured into water (50 mL) and the aqueous layer was extracted with methylene chloride (3x20 mL). The combined organic extracts were washed with brine (20 mL), dried over Na₂SO₄ and concentrated under reduced pressure. The residue was purified by flash chromatography (1:1= hexane: ethyl acetate with 1% isopropylamine) to afford the desired product (1.09 g, 94.3 % yield) as light-yellow solid: ¹H NMR (400 MHz, CDCl₃) δ 8.10 (s, 1H), 7.46-7.35 (m, 6H), 7.27 (m, 2H), 6.98 (apparent d, 1H, J=7.6 Hz), 5.02 (apparent dd, 1H, J=4.4, 8.1 Hz), 3.18 (apparent dd, 2H, J=2.5, 12.5 Hz), 2.74 (m, 2 H), 2.50 (m, 2H), 2.30-2.10 (m, 6H), 1.80 (m, 2H), 1.25 (d, 6H, J=7.1 Hz); ESMS m/e: 381.2 (M + H)⁺.

The hydrochloric salt was prepared by addition of a slight excess of 1 N HCl in ether (1.2 eq.) to a solution of the free base in dichloromethane. The solvent was removed under reduced pressure, the residue was washed with ether and dried under reduced pressure:

Anal. Calc. for C₂₄H₃₂N₂O₂+HCl+0.8H₂O: C, 66.82; H, 8.08; N, 6.49; Cl, 8.22. Found: C, 66.90; H, 7.78; N, 6.63; Cl, 8.52.

Method B

Into a 25-mL RB-flask was added (R)-(+)-3-chloro-1-phenyl-1-propanol (0.426 g, 2.50 mmol), 2-methyl-N-[3-(4-piperidinyl)phenyl]propanamide (0.565 g, 2.00 mmol), diisopropylethylamine (1.29 g, 10.0 mmol), dioxane (5.0 mL) and catalytic amount of tetrabutylammonium iodide at room temperature. After stirring at 90 °C for 72 hrs, the reaction mixture was poured into water (50 mL) and the aqueous layer was extracted with methylene chloride (3x20 mL). The combined organic extracts were washed

with brine (20 mL), dried over Na_2SO_4 and concentrated under reduced pressure. The residue was purified by preparative TLC plates (1:5:100=isopropylamine:methanol:ethyl acetate) to afford the desired product (0.260 g, 34.2 % yield) as light-yellow solid.

Example 114

N- (3-{1-[(3S)-3-(4-cyanophenoxy)-3-phenylpropyl]-4-piperidinyl}phenyl)-2-methylpropanamide
A mixture of N- (3-{1-[(3R)-3-hydroxy-3-phenylpropyl]-4-piperidinyl}phenyl)-2-methylpropanamide (5.20 mg, 0.0137 mmol), 4-cyanophenol (100 mg), triphenylphosphine (30.0 mg, 0.115 mmol) and diethyl azodicarboxylate (7.42 mg, 0.0426 mmol) in THF (0.50 mL) was stirred at room temperature for 3 days. Chromatography using silica preparative TLC plates [2.5% of NH_3 (2.0 M in methanol) in CHCl_3] gave the desired product (4.70 mg, 71.3 % yield) as a thick oil: ^1H NMR (400 MHz, CDCl_3) δ 7.54 (m, 2H), 7.48 (d, 2H, $J=8.4$ Hz), 7.30-7.20 (m, 3H), 7.20 (m, 3H), 6.97 (apparent d, 1H, $J=8.4$ Hz), 6.92 (apparent d, 2H, $J=8.4$ Hz), 5.36 (apparent dd, 1H, $J=3.9, 7.6$ Hz), 3.12 (m, 2H), 2.61 (m, 2H), 2.53 (apparent sept, partially hidden, 2H, $J=7.6$ Hz), 2.30-2.10 (m, 6H), 1.82 (m, 2H), 1.25 (d, 6H, $J=6.8$ Hz); ESMS m/e : 482.2 ($M + H$)⁺.

Example 115

N- (3-{1-[(3S)-3-(4-FLUOROPHOXY)-3-PHENYLPROPYL]-4-PIPERIDINYL}PHENYL)-2-METHYLPROPANAMIDE
A mixture of N- (3-{1-[(3R)-3-hydroxy-3-phenylpropyl]-4-piperidinyl}phenyl)-2-methylpropanamide (5.20 mg, 0.0137 mmol), 4-fluorophenol (100 mg), triphenylphosphine (30.0

mg, 0.115 mmol) and diethyl azodicarboxylate (7.42 mg, 0.0426 mmol) in THF (0.50 mL) was stirred at room temperature for 3 days. Chromatography using silica preparative TLC plates [2.5% of NH₃ (2.0 M in methanol) in CHCl₃] gave the desired product (4.20 mg, 64.7% yield) as a thick oil: ¹H NMR (400 MHz, CDCl₃) δ 7.40 (m, 2H), 7.30-7.20 (m, 5H), 7.20 (m, 3H), 6.97 (apparent d, 1H, J=7.7 Hz), 6.87 (m, 1H), 6.76 (m, 1H), 5.26 (apparent dd, 1H, J=4.0, 8.1 Hz), 3.09 (m, 2H), 2.66 (m, 2H), 2.51 (m, 2H), 2.3-2.1 (m, 6H), 1.82 (m, 2H), 1.25 (d, 6H, overlapped); ESMS m/e: 475.2 (M + H)⁺.

Example 116

N-(3-{1-[(3S)-3-(4-BROMOPHOXY)-3-PHENYLPROPYL]-4-PIPERIDINYLPHENYL}-2-METHYLPROPANAMIDE

A mixture of N-(3-{1-[(3R)-3-hydroxy-3-phenylpropyl]-4-piperidinyl}phenyl)-2-methylpropanamide (5.20 mg, 0.0137 mmol), 4-bromophenol (100 mg), triphenylphosphine (30.0 mg, 0.115 mmol) and diethyl azodicarboxylate (7.42 mg, 0.0426 mmol) in THF (0.50 mL) was stirred at room temperature for 3 days. Chromatography using silica preparative TLC plates [2.5% of NH₃ (2.0 M in methanol) in CHCl₃] the desired product (0.70 mg, 9.6% yield) as a thick oil: ¹H NMR (400 MHz, CDCl₃) δ 8.06 (s, 1H), 7.48 (m, 2H), 7.30-7.20 (m, 5H), 7.20 (m, 3H), 6.97 (apparent d, 1H, J=8.5 Hz), 6.73 (apparent d, 2H, J=8.5 Hz), 5.22 (apparent dd, 1H, J=4.9, 7.8 Hz), 3.15 (m, 2H), 2.65 (m, 2H), 2.51 (apparent sept, partially hidden, 2H, J=7.6 Hz), 2.30-2.10 (m, 6H), 1.82 (m, 2H), 1.25 (d, 6H, J=6.8 Hz); ESMS m/e: 535.1 (M + H)⁺.

Example 117

N-(3-{1-[(3S)-3-(3-METHOXYPHENOXY)-3-PHENYLPROPYL]-4-

PIPERIDINYL}PHENYL)-2-METHYLPROPANAMIDE

A mixture of N-(3-{1-[(3R)-3-hydroxy-3-phenylpropyl]-4-piperidinyl}phenyl)-2-methylpropanamide (5.20 mg, 0.0137 mmol), 3-methoxyphenol (100 mg), triphenylphosphine (30.0 mg, 0.115 mmol) and diethyl azodicarboxylate (7.42 mg, 0.0426 mmol) in THF (0.50 mL) was stirred at room temperature for 3 days. Chromatography using silica preparative TLC plates [2.5% of NH₃ (2.0 M in methanol) in CHCl₃] gave the desired product (3.1 mg, 46.6 % yield) as a thick oil: ¹H NMR (400 MHz, CDCl₃) δ 7.47 (d, 1H, J=6.7 Hz), 7.42 (s, 1H), 7.3-7.20 (m, 3H), 7.20 (m, 3H), 7.07 (t, 1H, J=8.4 Hz), 6.97 (apparent d, 1H, J=6.7 Hz), 6.40 (m, 3H), 5.27 (apparent dd, 1H, J=5.3, 8.0 Hz), 3.74 (s, 3H), 3.38 (m, 2H), 2.93 (m, 2H), 2.61 (s, 1H), 2.53 (apparent sept, partially hidden, 1H, J=6.5 Hz), 2.30-2.10 (m, 6H), 1.82 (m, 2H), 1.25 (d, 6H, J=6.9 Hz); ESMS m/e: 487.3 (M + H)⁺.

20 Example 118

N-(3-{1-[(3S)-3-(4-CYANO-2-METHOXYPHENOXY)-3-

PHENYLPROPYL]-4-PIPERIDINYL}PHENYL)-2-METHYLPROPANAMIDE

A mixture of N-(3-{1-[(3R)-3-hydroxy-3-phenylpropyl]-4-piperidinyl}phenyl)-2-methylpropanamide (5.20 mg, 0.0137 mmol), 2-methoxy-4-cyanophenol (100 mg), triphenylphosphine (30.0 mg, 0.115 mmol) and diethyl azodicarboxylate (7.42 mg, 0.0426 mmol) in THF (0.50 mL) was stirred at room temperature for 3 days. Chromatography using silica preparative TLC plates [2.5% of NH₃ (2.0 M in methanol) in CHCl₃] gave the desired product (5.50 mg, 76.5 % yield) as a thick oil: ¹H NMR (400 MHz, CDCl₃) δ 7.51 (s, 1H), 7.38 (s, 1H), 7.37 (d, 2H, J=2.4 Hz), 7.20 (m, 4H), 7.10 (d, 1H, J=2.4 Hz),

7.08 (s, 1H), 6.99 (apparent d, 1H, J=8.3 Hz), 6.76
5 (apparent d, 1H, J=8.3 Hz), 5.43 (apparent dd, 1H,
J=5.1, 8.0 Hz), 3.91 (s, 3H), 3.34 (m, 2H), 2.63 (m,
2H), 2.63 (s, 1H), 2.53 (apparent sept, partially
hidden, 1H, J=7.7 Hz), 2.30-2.10 (m, 6H), 1.82 (m, 2H),
1.28 (d, 6H, J=6.8 Hz); ESMS m/e: 512.2 (M + H)⁺.

Example 119

N-(3-{1-[(3S)-3-(5-ACETYL-2-METHOXYPHOXY)-3-PHENYLPROPYL]-4-PIPERIDINYL}PHENYL)-2-METHYLPROPANAMIDE
A mixture of N-(3-{1-[(3R)-3-hydroxy-3-phenylpropyl]-4-piperidinyl}phenyl)-2-methylpropanamide (5.20 mg, 0.0137 mmol), 2-methoxy-5-acetylphenol (100 mg), triphenylphosphine (30.0 mg, 0.115 mmol) and diethyl azodicarboxylate (7.42 mg, 0.0426 mmol) in THF (0.50 mL) was stirred at room temperature for 3 days.
Chromatography using silica preparative TLC plates [2.5% of NH₃ (2.0 M in methanol) in CHCl₃] gave the desired product (1.60 mg, 22.2 % yield) as a thick oil: ¹H NMR (400 MHz, CDCl₃) δ 7.52 (d, 2H, J=2.4 Hz), 7.3-7.2 (m, 5H), 7.20 (m, 3H), 6.97 (apparent d, 1H, J=6.7 Hz), 6.69 (apparent d, 1H, J=8.0 Hz), 5.47 (apparent dd, 1H, J=4.3, 7.8 Hz), 3.95 (s, 3H), 3.38 (m, 2H), 2.93 (m, 2H), 2.61 (s, 1H), 2.53 (apparent sept, partially hidden, 1H, J=7.6 Hz), 2.50 (s, 3H), 2.30-2.10 (m, 6H), 1.82 (m, 2H), 1.25 (d, 6H, J=6.8 Hz); ESMS m/e: 529.6 (M + H)⁺.

Example 120

N-(3-{1-[(3R)-3-(2-ACETYLPHENOXY)-3-PHENYLPROPYL]-4-PIPERIDINYL}PHENYL)-2-METHYLPROPANAMIDE
A mixture of N-(3-{1-[(3S)-3-hydroxy-3-phenylpropyl]-4-piperidinyl}phenyl)-2-methylpropanamide (5.2 mg, 0.0137

mmol), 2-acetylphenol (100 mg), triphenylphosphine (30.0 mg, 0.115 mmol) and diethyl azodicarboxylate (7.42 mg, 0.0426 mmol) in THF (0.50 mL) was stirred at room temperature for 3 days. Chromatography using silica preparative TLC plates [2.5% of NH₃ (2.0 M in methanol) in CHCl₃] gave the desired product (1.70 mg, 24.9 % yield) as a thick oil: ¹H NMR (400 MHz, CDCl₃) δ 7.65 (m, 1H), 7.55 (s, 1H), 7.30-7.20 (m, 5H), 7.20 (m, 3H), 6.97 (m, 2H), 6.76 (apparent d, 1H), 5.49 (apparent dd, 1H, J=4.3, 8.0 Hz), 3.38 (m, 2H), 2.93 (m, 2H), 2.71 (s, 3H), 2.60 (s, 1H), 2.53 (apparent sept, partially hidden, 1H, J=7.6 Hz), 2.30-2.10 (m, 6H), 1.82 (m, 2H), 1.25 (d, 6H, J=6.9 Hz); ESMS m/e: 498.8 (M⁺).

15 **Example 121**

N-[3-(1-{(3R)-3-[2-FLUORO-5-(TRIFLUOROMETHYL)PHENOXY]-3-PHENYLPROPYL}-4-PIPERIDINYL)PHENYL]-2-METHYLPROPANAMIDE

A mixture of N-(3-{1-[(3S)-3-hydroxy-3-phenylpropyl]-4-piperidinyl}phenyl)-2-methylpropanamide (5.20 mg, 0.0137 mmol), 2-fluoro-5-trifluoromethylphenol (100 mg), triphenylphosphine (30.0 mg, 0.115 mmol) and diethyl azodicarboxylate (7.42 mg, 0.0426 mmol) in THF (0.50 mL) was stirred at room temperature for 3 days.

Chromatography using silica preparative TLC plates [2.5% of NH₃ (2.0 M in methanol) in CHCl₃] gave the desired product (2.50 mg, 33.7 % yield) as a thick oil: ¹H NMR (400 MHz, CDCl₃) δ 8.07 (s, 1H), 7.67 (m, 1H), 7.54 (m, 1H), 7.45 (m, 2H), 7.30-7.10 (m, 6H), 7.14 (d, 1H, J=7.4 Hz), 6.97 (apparent d, 1H, J=7.7 Hz), 5.37 (apparent dd, 1H, J=5.0, 8.5 Hz), 3.4 (m, 2H), 2.8 (m, 2H), 2.6 (s, 1H), 2.53 (apparent sept, partially hidden, 1H, J=7.4 Hz), 2.30-2.10 (m, 6H), 1.80 (m, 2H), 1.25 (d, 6H, J=7.1 Hz, overlapped); ESMS m/e: 542.6 (M⁺), 543.54 (M + H)⁺.

Example 122

N-[3-(1-{(3S)-3-[2-FLUORO-5-(TRIFLUOROMETHYL)PHENOXY]-3-PHENYLPROPYL}-4-PIPERIDINYL)PHENYL]-2-METHYLPROPANAMIDE

5 A mixture of N-(3-{1-[(3R)-3-hydroxy-3-phenylpropyl]-4-piperidinyl}phenyl)-2-methylpropanamide (5.20 mg, 0.0137 mmol), 2-fluoro-5-trifluoromethylphenol (100 mg), triphenylphosphine (30.0 mg, 0.115 mmol) and diethyl azodicarboxylate (7.42 mg, 0.0426 mmol) in THF (0.50 mL)

10 was stirred at room temperature for 3 days.

Chromatography using silica preparative TLC plates [2.5% of NH₃ (2.0 M in methanol) in CHCl₃] gave the desired product (3.00 mg, 40.4% yield) as a thick oil: ¹H NMR (400 MHz, CDCl₃) δ 8.06 (s, 1H), 7.67 (m, 2H), 7.55 (m, 2H), 7.50-7.40 (m, 3H), 7.30-7.10 (m, 3H), 7.17 (d, 1H, J=8.9 Hz), 7.07 (apparent d, 1H, J=6.7 Hz), 6.97 (apparent d, 1H, J=7.8 Hz), 5.37 (apparent dd, 1H, J=4.2, 8.1 Hz), 3.37 (m, 2H), 2.93 (m, 2H), 2.63 (s, 1H), 2.50 (apparent sept, partially hidden, 1H, J=7.9 Hz), 2.30-2.10 (m, 6H), 1.85 (m, 2H), 1.25 (d, 6H, J=6.9 Hz); ESMS m/e: 542.7 (M + H)⁺.

Example 123

N-(3-{1-[(3S)-3-(2,5-DIFLUOROPHOXY)-3-PHENYLPROPYL]-4-PIPERIDINYL)PHENYL]-2-METHYLPROPANAMIDE

25 A mixture of N-(3-{1-[(3R)-3-hydroxy-3-phenylpropyl]-4-piperidinyl}phenyl)-2-methylpropanamide (5.20 mg, 0.0137 mmol), 2,5-difluorophenol (100 mg), triphenylphosphine (30.0 mg, 0.115 mmol) and diethyl azodicarboxylate (7.42 mg, 0.0426 mmol) in THF (0.50 mL) was stirred at room temperature for 3 days. Chromatography using silica preparative TLC plates [2.5% of NH₃ (2.0 M in methanol) in CHCl₃] gave the desired product (2.70 mg, 40.1 %

yield) as a thick oil: ^1H NMR (400 MHz, CDCl_3) δ 7.46 (s, 1H), 7.40-7.30 (m, 4H), 7.20 (m, 2H), 7.17 (s, 1H), 6.97 (m, 2H), 6.58 (m, 1H), 6.51 (m, 1H), 5.27 (apparent dd, 1H, $J=5.1$, 8.2 Hz), 3.13 (apparent d, $J=9.7$ Hz, 2H), 2.64 (m, 2H), 2.51 (m, 2H), 2.34 (apparent sept, partially hidden, $J=7.1$ Hz, 1H), 2.17 (m, 3H), 1.90-1.80 (m, 4H), 1.25 (d, 6H, $J=7.1$ Hz); ESMS m/e : 493.1 ($M + H$)⁺.

10 **Example 124**

N-(3-{1-[(3R)-3-(3-CHLOROPHOXY)-3-PHENYLPROPYL]-4-PIPERIDINYLPHENYL}-2-METHYLPROPANAMIDE

A mixture of N-(3-{1-[(3S)-3-hydroxy-3-phenylpropyl]-4-piperidinyl}phenyl)-2-methylpropanamide (5.20 mg, 0.0137 mmol), 3-chlorophenol (100 mg), triphenylphosphine (30.0 mg, 0.115 mmol) and diethyl azodicarboxylate (7.42 mg, 0.0426 mmol) in THF (0.50 mL) was stirred at room temperature for 3 days. Chromatography using silica preparative TLC plates [2.5% of NH_3 (2.0 M in methanol) in CHCl_3] gave the desired product (2.4 mg, 35.8% yield) as a thick oil: ^1H NMR (400 MHz, CDCl_3) δ 7.30 (m, 2H), 7.30-7.20 (m, 3H), 7.20 (m, 3H), 6.90 (apparent d, 1H, $J=7.7$ Hz), 6.71 (apparent d, 1H, $J=2.9$ Hz), 6.69 (apparent t, 1H, $J=2.9$ Hz), 6.67 (apparent t, 1H, $J=2.9$ Hz), 6.65 (apparent d, 1H, $J=2.9$ Hz), 5.09 (apparent dd, 1H, $J=4.8$, 8.1 Hz), 3.18 (m, 2H), 2.73 (m, 2H), 2.50 (apparent sept, partially hidden, 2H, $J=7.1$ Hz), 2.30-2.10 (m, 6H), 1.89 (m, 2H), 1.25 (d, 6H, overlapped); ESMS m/e : 491.1 ($M + H$)⁺.

30

Example 125

(1S)-3-{4-[3-(ISOBUTYRYLAMINO)PHENYL]-1-PIPERIDINYL}-1-

Into a 25-mL RB-flask was added N-(3-{1-[(3S)-3-hydroxy-3-phenylpropyl]-4-piperidinyl}phenyl)-2-methylpropanamide (5.20 mg, 0.0137 mmol), 1-naphthalenecarbonyl chloride (100 mg),
5 diisopropylethylamine (0.30 mL) in THF (0.50 mL) at room temperature. After stirring for 16 hrs at room temperature, the reaction mixture was concentrated under reduced pressure. The residue was purified using preparative TLC plates [2.5% of NH₃ (2.0 M in methanol)
10 in CHCl₃] gave the desired product (4.70 mg, 71.3 % yield) as a thick oil: ¹H NMR (400 MHz, CDCl₃) δ 8.90 (d, 1H, J=8.9 Hz), 8.28 (apparent dd, 1H, J=1.5, 7.2 Hz), 8.03 (d, 1H, J=8.7 Hz), 7.88 (dm, 2H, J=8.7 Hz), 7.60-7.48 (m, 7H), 7.40-7.32 (m, 3H), 7.25 (m, 1H), 6.90 (apparent d, 1H, J=7.4 Hz), 6.18 (apparent dd, 1H, J=5.7, 7.8 Hz), 3.42 (m, 2H), 2.84 (m, 2H), 2.53 (m, 2H), 2.44 (apparent sept, partially hidden, 4H, J=7.5 Hz), 2.30-2.10 (m, 2H), 1.82 (m, 2H), 1.25 (d, 6H, J=6.8 Hz); ESMS m/e: 535.6 (M + H)⁺.

20

Example 126

N-(3-{1-[(3S)-3-(3-ACETYLPHENOXY)-3-PHENYLPROPYL]-4-PIPERIDINYL}PHENYL)-2-METHYLPROPANAMIDE

A mixture of N-(3-{1-[(3R)-3-hydroxy-3-phenylpropyl]-4-piperidinyl}phenyl)-2-methylpropanamide (5.20 mg, 0.0137 mmol), 2-acetylphenol (100 mg), triphenylphosphine (30.0 mg, 0.115 mmol) and diethyl azodicarboxylate (7.42 mg, 0.0426 mmol) in THF (0.50 mL) was stirred at room temperature for 3 days. Chromatography using silica preparative TLC plates [2.5% of NH₃ (2.0 M in methanol)
25 in CHCl₃] gave the desired product (1.50 mg, 22.0% yield) as a thick oil: ¹H NMR (400 MHz, CDCl₃) δ 7.65 (m, 1H), 7.55 (s, 1H), 7.30-7.20 (m, 5H), 7.20 (m, 3H), 6.97 (m,
30 6.85, 1H), 6.75 (s, 1H), 6.65 (s, 1H), 6.55 (s, 1H), 6.45 (s, 1H), 6.35 (s, 1H), 6.25 (s, 1H), 6.15 (s, 1H), 6.05 (s, 1H), 5.95 (s, 1H), 5.85 (s, 1H), 5.75 (s, 1H), 5.65 (s, 1H), 5.55 (s, 1H), 5.45 (s, 1H), 5.35 (s, 1H), 5.25 (s, 1H), 5.15 (s, 1H), 5.05 (s, 1H), 4.95 (s, 1H), 4.85 (s, 1H), 4.75 (s, 1H), 4.65 (s, 1H), 4.55 (s, 1H), 4.45 (s, 1H), 4.35 (s, 1H), 4.25 (s, 1H), 4.15 (s, 1H), 4.05 (s, 1H), 3.95 (s, 1H), 3.85 (s, 1H), 3.75 (s, 1H), 3.65 (s, 1H), 3.55 (s, 1H), 3.45 (s, 1H), 3.35 (s, 1H), 3.25 (s, 1H), 3.15 (s, 1H), 3.05 (s, 1H), 2.95 (s, 1H), 2.85 (s, 1H), 2.75 (s, 1H), 2.65 (s, 1H), 2.55 (s, 1H), 2.45 (s, 1H), 2.35 (s, 1H), 2.25 (s, 1H), 2.15 (s, 1H), 2.05 (s, 1H), 1.95 (s, 1H), 1.85 (s, 1H), 1.75 (s, 1H), 1.65 (s, 1H), 1.55 (s, 1H), 1.45 (s, 1H), 1.35 (s, 1H), 1.25 (s, 1H), 1.15 (s, 1H), 1.05 (s, 1H), 0.95 (s, 1H), 0.85 (s, 1H), 0.75 (s, 1H), 0.65 (s, 1H), 0.55 (s, 1H), 0.45 (s, 1H), 0.35 (s, 1H), 0.25 (s, 1H), 0.15 (s, 1H), 0.05 (s, 1H), -0.05 (s, 1H); ESMS m/e: 535.6 (M + H)⁺.

2H), 6.76 (apparent d, 1H), 5.49 (apparent dd, 1H, J=4.3, 8.0 Hz), 3.38 (m, 2H), 2.93 (m, 2H), 2.75 (s, 3H), 2.53 (apparent sept, partially hidden, 2H, J=7.6 Hz), 2.30-2.10 (m, 6H), 1.92 (m, 2H), 1.25 (d, 6H, J=6.9 Hz); ESMS m/e: 498.81 (M⁺), 499.6 (M + H)⁺.

Example 127

N-(3-{1-[(3S)-3-(2-FLUOROPHOXY)-3-PHENYLPROPYL]-4-PIPERIDINYL}PHENYL)-2-METHYLPROPANAMIDE

A mixture of N-(3-{1-[(3R)-3-hydroxy-3-phenylpropyl]-4-piperidinyl}phenyl)-2-methylpropanamide (5.20 mg, 0.0137 mmol), 2-fluorophenol (100 mg), triphenylphosphine (30.0 mg, 0.115 mmol) and diethyl azodicarboxylate (7.42 mg, 0.0426 mmol) in THF (0.50 mL) was stirred at room temperature for 3 days. Chromatography using silica preparative TLC plates [2.5% of NH₃ (2.0 M in methanol) in CHCl₃] gave the desired product (3.5 mg, 53.9% yield) as a thick oil: ¹H NMR (400 MHz, CDCl₃) δ 8.07 (s, 1H), 7.65 (m, 1H), 7.41 (s, 1H), 7.40-7.10 (m, 5H), 7.05 (m, 2H), 6.97 (apparent d, 1H, J=8.7 Hz), 6.86 (m, 2H), 6.79 (apparent dt, 1H, J=2.4, 7.9 Hz), 5.31 (apparent dd, 1H, J=4.5, 8.0 Hz), 3.39 (m, 2H), 2.97 (m, 2H), 2.53 (apparent sept, partially hidden, 2H, J=7.5 Hz), 2.3-2.1 (m, 6H), 1.92 (m, 2H), 1.25 (d, 6H, J=6.7 Hz); ESMS m/e: 475.7 (M + H)⁺.

Example 128

(4S)-N-(3-{4-[3-(ACETYLAMINO)PHENYL]-1-PIPERIDINYL}PROPYL)-4-(3,5-DIFLUOROPHENYL)-2-OXO-1,3-OXAZOLIDINE-3-CARBOXAMIDE

30

Method:

Into a 20 ml vial was added N1-{3-[1-(aminopropyl)-
1,2,3,6-tetrahydro-4-pyridinyl]phenyl}acetamide (15 mg,
0.054 mmol), 4-(3,5-Difluorophenyl)-2-oxo-oxazolidine-3-
carboxylic acid-4-nitro-phenyl ester (39.3 mg, 1.08
mmol, 2 eq) and dichloromethane with 0.6% of Methanol (3
ml) at room temperature. After stirring at room
temperature for 3 hrs, the reaction mixture was
filtered, and purified by preparative silica TLC (19:1 =
chloroform : methanol) to afford the desired product
(18.3 mg, 68% yield); ^1H NMR (400 MHz, CDCl_3) δ 8.09 (br
s, 1H), 7.40 (d, 1H, $J=8.0$ Hz), 7.36-7.28 (m, 2H), 7.24
(t, 1H, $J=8.0$ Hz), 6.99 (d, 1H, $J=8.0$ Hz), 6.86-6.82 (m,
2H), 5.41 (dd, 1H, $J=4.1, 9.0$ Hz), 4.72 (t, 1H, $J=9.0$
Hz), 4.22 (dd, 1H, $J=3.9, 9.1$ Hz), 3.42-3.29 (m, 2H),
3.02 (d, 2H $J=11.1$ Hz), 2.52-2.38 (m, 3H), 2.16 (s, 3H),
2.08-1.98 (m, 2H), 1.86-1.70 (m, 6H); ESMS m/e: 501.2 (M
+ H) $^+$; Anal. Calc. for $\text{C}_{26}\text{H}_{30}\text{F}_2\text{N}_4\text{O}_4 + 0.5\text{H}_2\text{O}$: C, 60.64; H,
6.18; N, 10.88. Found: C, 60.67; H, 5.79; N, 10.86.

20 **Example 129**

The synthetic method is the same as described for the
synthesis of (4S)-N-(3-{4-[3-(acetylamino)phenyl]-1-
piperidinyl}propyl)-4-(3,5-difluorophenyl)-2-oxo-1,3-
oxazolidine-3-carboxamide.

25

**(4S)-N-(3-{4-[3-(ACETYLAMINO) PHENYL]-1-
PIPERIDINYL}PROPYL)-2-OXO-4-(3,4,5-TRIFLUOROPHENYL)-1,3-
OXAZOLIDINE-3-CARBOXAMIDE:** 18.8 mg (67% yield); ^1H NMR
(400 MHz, CDCl_3) δ 8.09 (br s, 1H), 7.41-7.20 (m, 3H),
7.02-6.91 (m, 3H), 5.37 (dd, 1H, $J=3.8, 8.9$ Hz), 4.71
(t, 1H, $J=9$ Hz), 4.21 (dd, 1H, $J=4, 9.3$ Hz), 3.43-3.27
(m, 2H), 3.02 (d, 2H, $J=11.0$ Hz), 2.53-2.37 (m, 3H),
2.16 (s, 3H), 2.08-1.97 (m, 2H), 1.85-1.69 (m, 6H); ESMS

m/e: 519.2 ($M + H$)⁺; Anal. Calc. for $C_{26}H_{29}F_3N_4O_4 + 0.5H_2O$: C, 59.20; H, 5.73; N, 10.62. Found: C, 59.40; H, 5.35; N, 10.65.

5 **Example 130**

The synthetic method is the same as described for the synthesis of (4S)-N-(3-{4-[3-(acetylamino)phenyl]-1-piperidinyl}propyl)-4-(3,5-difluorophenyl)-2-oxo-1,3-oxazolidine-3-carboxamide.

10

N-(3-{4-[3-(ACETYLAMINO) PHENYL]-1-PIPERIDINYL}PROPYL)-4-(3,4-DIFLUOROPHENYL)-5,5-DIMETHYL-2-OXO-1,3-OXAZOLIDINE-3-CARBOXAMIDE: 19.6 mg (68% yield); 1H NMR (400 MHz, $CDCl_3$) δ 8.18 (t, 1H, $J=5.9$ Hz), 7.41 (d, 1H, $J=8.8$ Hz), 7.33 (s, 1H), 7.27-7.14 (m, 2H), 7.02-6.88 (m, 3H), 5.04 (s, 1H), 3.34 (qm, 2H, $J=6.3$ Hz), 3.02 (dm, 2H, $J=10.9$ Hz), 2.53-2.38 (m, 3H), 2.16 (s, 3H), 2.07-1.96 (m, 2H), 1.87-1.69 (m, 6H), 1.62 (s, 3H), 1.02 (s, 3H); ESMS m/e: 529.3 ($M + H$)⁺; Anal. Calc. for $C_{28}H_{34}F_2N_4O_4$: C, 63.62; H, 6.48; N, 10.60. Found: C, 63.15; H, 6.27; N, 10.48.

15

20

Example 131

The synthetic method is the same as described for the synthesis of (4S)-N-(3-{4-[3-(acetylamino)phenyl]-1-piperidinyl}propyl)-4-(3,5-difluorophenyl)-2-oxo-1,3-oxazolidine-3-carboxamide.

25

30

(4S,5R)-N-(3-{4-[3-(ACETYLAMINO) PHENYL]-1-PIPERIDINYL}PROPYL)-4-(3,4-DIFLUOROPHENYL)-5-METHYL-2-OXO-1,3-OXAZOLIDINE-3-CARBOXAMIDE: 20.5 mg (74% yield); 1H NMR (400 MHz, $CDCl_3$) δ 8.14 (t, 1H, $J=5.5$ Hz), 7.40 (d, 1H, $J=7.8$ Hz), 7.37-6.89 (m, 6H), 5.35 (d, 1H, $J=7.5$

Hz), 5.02-4.93 (m, 1H), 3.41-3.25 (m, 2H), 3.02 (d, 2H, J=10.8 Hz), 2.53-2.37 (m, 3H), 2.16 (s, 3H), 2.07 (m, 2H), 1.89-1.68 (m, 6H), 1.04 (d, 3H, J=6.4 Hz); ESMS m/e: 515.3 (M + H)⁺; Anal. Calc. for C₂₇H₃₂F₂N₄O₄+0.5H₂O: C, 61.94; H, 6.35; N, 10.70. Found: C, 61.90; H, 6.13; N, 10.64.

Example 132

The synthetic method is the same as described for the synthesis of (4S)-N-(3-{4-[3-(acetylamino)phenyl]-1-piperidinyl}propyl)-4-(3,5-difluorophenyl)-2-oxo-1,3-oxazolidine-3-carboxamide.

N-(3-{4-[3-(ACETYLAMINO)PHENYL]-1-PIPERIDINYL}PROPYL)-4-(4-FLUOROBENZYL)-2-OXO-1,3-OXAZOLIDINE-3-CARBOXAMIDE:
17.4 mg (65% yield); ¹H NMR (400 MHz, CDCl₃) δ 8.08 (t, 1H, J=5.6 Hz), 7.4 (d, 1H, J=7.2 Hz), 7.34 (s, 1H), 7.28-7.14 (m, 3H), 7.05-6.95 (m, 3H), 4.69-4.60 (m, 1H), 4.26 (t, 1H, J=8.8 Hz), 4.15 (dd, 1H, J=3.2, 9 Hz), 3.43 (q, 2H, J=6.2 Hz), 3.3 (dm 1H, J=13.6 Hz), 3.04 (dm, 2H, J=11 Hz), 2.87 (dd, 1H, J=9.3, 14.4 Hz), 2.53-2.42 (m, 3H), 2.16 (s, 3H), 2.09-1.99 (m, 2H), 1.87-1.65 (m, 6H); ESMS m/e: 497.3 (M + H)⁺; Anal. Calc. for C₂₇H₃₃FN₄O₄+0.5H₂O: C, 64.14; H, 6.78; N, 11.08. Found: C, 64.26; H, 6.39; N, 11.12.

Example 133

2-METHYL-N-(3-{1-[(3R)-3-(2-NITROPHENOXY)-3-PHENYLPROPYL]-4-PIPERIDINYL}PHENYL)PROPANAMIDE
A mixture of N-(3-{1-[(3S)-3-hydroxy-3-phenylpropyl]-4-piperidinyl}phenyl)-2-methylpropanamide (5.20 mg, 0.0137 mmol), 2-nitrophenol (100 mg), triphenylphosphine (30.0

mg, 0.115 mmol) and diethyl azodicarboxylate (7.42 mg, 0.0426 mmol) in THF (0.50 mL) was stirred at room temperature for 3 days. Chromatography using silica preparative TLC plates [2.5% of NH₃ (2.0 M in methanol) in CHCl₃] gave the desired product (2.37 mg, 34.5% yield) as a thick oil: ¹H NMR (400 MHz, CDCl₃) δ 7.84 (d, 1H), 7.90 (m, 1H), 7.45 (m 1H), 7.30-7.20 (m, 5H), 7.20 (m, 2H), 6.98 (m, 2H), 6.89 (apparent d, 1H, J=7.7 Hz), 5.62 (apparent dd, 1H, J=4.1, 8.9 Hz), 3.10 (m, 2H), 2.60 (m, 2H), 2.53 (m, 2H), 2.30-2.10 (m, 6H), 1.90 (m, 2H), 1.25 (d, 6H, overlapped); ESMS m/e: 502.3 (M + H)⁺.

Example 134

15 **N-(3-{1-[(3S)-3-([1,1'-BIPHENYL]-4-YLOXY)-3-PHENYLPROPYL]-4-PIPERIDINYL}PHENYL)-2-METHYLPROPANAMIDE**
A mixture of N-(3-{1-[(3R)-3-hydroxy-3-phenylpropyl]-4-piperidinyl}phenyl)-2-methylpropanamide (5.20 mg, 0.0137 mmol), 4-phenylphenol (100 mg), triphenylphosphine (30.0 mg, 0.115 mmol) and diethyl azodicarboxylate (7.42 mg, 0.0426 mmol) in THF (0.50 mL) was stirred at room temperature for 3 days. Chromatography using silica preparative TLC plates [2.5% of NH₃ (2.0 M in methanol) in CHCl₃] gave the desired product (3.00 mg, 41.2% yield) as a thick oil: ¹H NMR (400 MHz, CDCl₃) δ 8.06 (s, 1H), 7.48 (m, 2H), 7.40-7.30 (m, 8H), 7.30-7.25 (m, 4H), 6.97 (apparent d, 1H, J=7.6 Hz), 6.91 (apparent d, 2H, J=8.7 Hz), 5.34 (apparent dd, 1H, J=4.4, 8.0 Hz), 3.40 (m, 2H), 2.98 (m, 2H), 2.53 (apparent sept, partially hidden, 1H, J=8.1 Hz), 2.44 (m, 1H), 2.30-2.10 (m, 6H), 1.93 (d, 2H), 1.26 (d, 6H, J=6.9 Hz); ESMS m/e: 533.4 (M + H)⁺.

Example 135

2-METHYL-N-(3-{1-[*(3R*)-3-(3-NITROPHENOXY)-3-PHENYLPROPYL]-4-PIPERIDINYL}PHENYL)PROPANAMIDE

A mixture of N-(3-{1-[*(3S*)-3-hydroxy-3-phenylpropyl]-4-piperidinyl}phenyl)-2-methylpropanamide (5.20 mg, 0.0137 mmol), 3-nitrophenol (100 mg), triphenylphosphine (30.0 mg, 0.115 mmol) and diethyl azodicarboxylate (7.42 mg, 0.0426 mmol) in THF (0.50 mL) was stirred at room temperature for 3 days. Chromatography using silica preparative TLC plates [2.5% of NH₃ (2.0 M in methanol) in CHCl₃] gave the desired product (2.80 mg, 40.8 % yield) as a thick oil: ¹H NMR (400 MHz, CDCl₃) δ 7.76 (dm, 1H), 7.71 (t, 1H, J=1.8 Hz), 7.50-7.40 (m, 2H), 7.40-7.25 (m, 7H), 7.17 (apparent dd, 1H, J=2.4, 8.2), 6.97 (apparent d, 1H, J=7.7 Hz), 5.45 (apparent dd, 1H, J=5.0, 8.1 Hz), 3.45 (m, 2H), 2.89 (m, 2H), 2.53 (apparent sept, partially hidden, 2H, J=8.3 Hz), 2.30-2.10 (m, 6H), 1.92 (m, 2H), 1.25 (d, 6H, J=6.8 Hz); ESMS m/e: 502.3 (M + H)⁺.

20

Example 136

N-(3-{1-[*(3S*)-3-(2-ETHOXYPHENOXY)-3-PHENYLPROPYL]-4-PIPERIDINYL}PHENYL)-2-METHYLPROPANAMIDE

A mixture of N-(3-{1-[*(3R*)-3-hydroxy-3-phenylpropyl]-4-piperidinyl}phenyl)-2-methylpropanamide (5.20 mg, 0.0137 mmol), 2-ethoxyphenol (100 mg), triphenylphosphine (30.0 mg, 0.115 mmol) and diethyl azodicarboxylate (7.42 mg, 0.0426 mmol) in THF (0.50 mL) was stirred at room temperature for 3 days. Chromatography using silica preparative TLC plates [2.5% of NH₃ (2.0 M in methanol) in CHCl₃] gave the desired product (1.16 mg, 15.5% yield) as a thick oil: ¹H NMR (400 MHz, CDCl₃) δ 8.06 (s, 1H), 7.52 (s, 1H), 7.40-7.33 (m, 4H), 7.30-7.20 (m, 3H), 6.97

(apparent d, 1H, J=7.7 Hz), 6.88 (m, 2H), 6.68 (m, 2H),
5.21 (m, 1H), 4.11 (q, 2H, J=7.3 Hz), 3.37 (m, 2H), 2.71
(m, 2H), 2.53 (apparent sept, partially hidden, 2H,
J=7.6 Hz), 2.30-2.10 (m, 6H), 1.89 (m, 2H), 1.49 (t, 3H,
5 J=7.3 Hz), 1.25 (d, 6H, J=6.8 Hz); ESMS m/e: 501.4 (M +
H)⁺.

Example 137

2-METHYL-N-(3-{1-[(3S)-3-(1-NAPHTHYLOXY)-3-
10 PHENYLPROPYL]-4-PIPERIDINYL}PHENYL)PROPANAMIDE
A mixture of N-(3-{1-[(3R)-3-hydroxy-3-phenylpropyl]-4-
15 piperidinyl}phenyl)-2-methylpropanamide (5.20 mg, 0.0137
mmol), 1-naphthol (100 mg), triphenylphosphine (30.0 mg,
0.115 mmol) and diethyl azodicarboxylate (7.42 mg,
0.0426 mmol) in THF (0.50 mL) was stirred at room
temperature for 3 days. Chromatography using silica
20 preparative TLC plates [2.5% of NH₃ (2.0 M in methanol)
in CHCl₃] gave the desired product (4.30 mg, 66.2% yield)
as a thick oil: ¹H NMR (400 MHz, CDCl₃) δ 8.06 (s, 1H),
25 7.72 (d, 1H, J=8.5 Hz), 7.59 (d, 1H, J=8.5 Hz), 7.5 (m,
2H), 7.45-7.30 (m, 6H), 7.25 (m, 3H), 7.17 (apparent dd,
1H, J=2.6, 9.0 Hz), 7.01 (apparent d, 1H, J=2.6 Hz),
6.97 (apparent d, 1H, J=7.9 Hz), 5.46 (apparent dd, 1H,
J=4.5, 8.1 Hz), 3.12 (m, 2H), 2.61 (m, 2H), 2.53
2.30-
2.10 (m, 6H), 1.90 (m, 2H), 1.25 (d, 6H, J=7.3 Hz,
overlapped); ESMS m/e: 507.2 (M + H)⁺.

Example 138

30 N-(3-{1-[(3S)-3-(1,3-DIOXO-1,3-DIHYDRO-2H-ISOINDOL-2-
YL)-3-PHENYLPROPYL]-4-PIPERIDINYL}PHENYL)-2-
METHYLPROPANAMIDE

Step 1:

2-[(1S)-3-CHLORO-1-PHENYLPROPYL]-1H-ISOINDOLE-1,3(2H)-

DIONE:

A mixture of phthalimide (0.147 g, 1.0 mmol), (R)-(+)-3-chloro-phenyl-1-propanol (0.171 g, 1.0 mmol), triphenylphosphine (0.262 g, 1.0 mmol), diethyl azodicarboxylate (0.174 g, 1.0 mmol) in 5.0 mL of THF

10 was stirred at room temperature for 24 h. The reaction mixture was concentrated *in vacuo*. The residue was washed with pentane (x3) and the combined pentane extracts were concentrated and chromatographed (silica with hexanes-EtOAc 8:1 as the eluent) to give the

15 desired product (as described as a general procedure by: Srebnik, M.; Ramachandran, P.V.; Brown, H.C. *J. Org. Chem.* 1988, 53, 2916-2920) afforded the desired product (0.121 g, 50.2 %) as a yellow solid: ^1H NMR (400 MHz, CDCl₃) δ 7.82 (apparent dd, 2H, J=2.9 Hz), 7.70 (apparent dd, 2H, J=2.9 Hz), 7.56 (m, 2H), 7.39-7.27 (m, 3H), 5.64 (apparent dd, 1H, J=7.0, 9.2 Hz), 3.57 (m, 2H), 3.05 (m, 1H), 2.82 (apparent sept, 1H, J=7.0 Hz); ESMS *m/e*: 300.13 (M+H)⁺.

25 Step 2:

N-(3-{1-[(3S)-3-(1,3-DIOXO-1,3-DIHYDRO-2H-ISOINDOL-2-

YL)-3-PHENYLPROPYL]-4-PIPERIDINYL}PHENYL)-2-

METHYLPROPANAMIDE: A mixture of potassium carbonate

30 (29.2 mg, 0.211 mmol), sodium iodide (47.5 mg, 0.317 mmol), 2-methyl-*N*-(3-(4-piperidinyl)phenyl)propanamide (51.8 mg, 0.211 mmol) 2-[(1S)-3-chloro-1-phenylpropyl]-1H-isoindole-1,3(2H)-dione

(63.1 mg, 0.211 mmol) in DMF (5.0 mL) was stirred at 100 °C for 3 hrs, at which time TLC indicated that the reaction was complete. The reaction mixture was poured into water (50 mL) and the aqueous layer was extracted with methylene chloride (3x30 mL). The combined organic extracts were washed with brine (30 mL), dried over MgSO₄ and concentrated under reduced pressure. The crude product was purified by Prep-TLC plates [2.5% of NH₃ (2.0 M in methanol) in CHCl₃]
to give the desired product (74.1 mg, 77.1 %) as a thick oil: ¹H NMR (400 MHz, CDCl₃) δ 7.83 (apparent dd, 2H, J=2.9 Hz), 7.69 (apparent dd, 2H, J=2.9 Hz), 7.56 (apparent dt, 3H, J=2.9, 7.3 Hz), 7.33 (m, 4H), 7.21 (t, 1H, J=7.8 Hz), 7.09 (s, 1H), 6.81 (apparent d, 1H, J=7.8 Hz), 5.49 (apparent dd, 1H, J=5.5, 9.5 Hz), 2.98 (d, 1H, J=9.5 Hz), 2.87 (m, 2H), 2.50 (apparent sept, 1H, J=6.7 Hz), 2.40-2.35 (m, 4H), 1.94 (m, 2H), 1.70-1.50 (m, 4H), 1.25 (d, 6H, J=7.9 Hz); ESMS m/e: 510.37 (M+H)⁺.

**Example 139
2-METHYL-N-(3-{1-[*(3S*)-3-(4-PHOXYPHENOXY)-3-PHENYLPROPYL]-4-PIPERIDINYL}PHENYL)PROPANAMIDE**

STEP 1:

4-{[(1*S*)-3-CHLORO-1-PHENYLPROPYL]OXY}-(4-PHOXY)BENZENE:
A mixture of 4-phenoxyphenol (1.86 g, 10.0 mmol), (*S*)-(−)-3-chloro-phenyl-1-propanol (1.70 g, 10.0 mmol), triphenylphosphine (2.62 g, 10.0 mmol), diethyl azodicarboxylate (1.57 mL, 10.0 mmol) in 5.0 mL of THF was stirred at room temperature for 24 h. The reaction mixture was concentrated *in vacuo*. The residue was

washed with pentane (x3) and the combined pentane extracts were concentrated and chromatographed (silica with hexanes-EtOAc 97:3 as the eluent) to give the desired product (as described as a general procedure by:
5 Srebnik, M.; Ramachandran, P.V.; Brown, H.C. *J. Org. Chem.* 1988, 53, 2916-2920) afforded the desired product as a thick oil which solidified on standing (2.51 g, 75.7 %): ^1H NMR (400 MHz, CDCl_3) δ 7.4-7.23 (m, 7H), 7.03 (apparent t, 1H, $J=7.3$ Hz), 6.91 (apparent dm, 2H, $J=7.8$ Hz), 6.93 (apparent q, 4H, $J=7.8$ Hz), 5.31 (apparent dd, 1H, $J=4.5$, 8.6 Hz), 3.82 (m, 1H), 3.62 (apparent quintet, 1H, $J=5.6$ Hz), 2.47 (m, 1H), 2.20 (m, 1H).

Step 2:

15 **2-METHYL-N-[(3-[1-(3S)-3-(4-PHOXYPHENOXY)-3-PHENYLPROPYL]-4-PIPERIDINYL)PHENYL]PROPANAMIDE:** A mixture of 2-methyl-N-[3-(4-piperidinyl)phenyl]propanamide (65.5 mg, 0.266 mmol), 4-{[(1S)-3-chloro-1-phenylpropyl]oxy}-(4-phenoxy)benzene (0.100 mg, 0.296 mmol), potassium carbonate (40.9 mg, 0.296 mmol) and sodium iodide (67.0 mg, 0.444 mmol) in DMF (1.0 mL) at 100 °C for 3 hours. The reaction mixture was poured into water (50 mL) and the aqueous layer was extracted with methylene chloride (3x30 mL). The combined organic extracts were washed with brine (30 mL), dried over MgSO_4 and concentrated under reduced pressure. The crude product was purified by Prep-TLC plates [2.5% of NH_3 (2.0 M in methanol) in CHCl_3] to give the desired product (0.109 g, 74.6 %) as a thick oil: ^1H NMR (400 MHz, CDCl_3) δ 7.48 (s, 1H), 7.40-7.30 (m, 4H), 7.20-7.10 (m, 6 H), 7.09 (s, 1H), 6.99 (apparent d, 1H, $J=7.8$ Hz), 6.98 (apparent t, 1H, $J=7.8$ Hz), 6.93

(apparent d, 2H, J=8.4 Hz), 6.84 (m, 2H), 5.20 (apparent dd, 1H, J=4.4, 8.5 Hz), 3.03 (m, 2H), 2.51 (m, 4H), 2.24 (apparent sept, 1H, J=7.8 Hz), 2.20-2.10 (m, 3H), 1.90 (m, 4H), 1.25 (d, 6H, J=7.9 Hz); ESMS m/e: 549.41 (M+H)⁺;
5 Anal. Calc. for C₃₆H₄₀N₂O₃: C, 78.80; H, 7.35; N, 5.11.
Found: C, 78.58; H, 7.48; N, 5.09.

Example 140

10 **N- (4-{1-[(3R)-3-(3,4-DIMETHOXYPHOXY)-3-PHENYLPROPYL]-4-PIPERIDINYL}PHENYL)-2-METHYLPROPANAMIDE**

Step 1:

15 **1-[(3R)-3-(3,4-DIMETHOXYPHOXY)-3-PHENYLPROPYL]-4-(4-NITROPHENYL)-1,2,3,6-TETRAHYDROPYRIDINE:**

A mixture of potassium carbonate (24.0 mg, 0.174 mmol), sodium iodide (39.0 mg, 0.260 mmol), 4-(4-nitrophenyl)-1,2,3,6-tetrahydropyridine (35.4 mg, 0.174 mmol) and 4-{[(1R)-3-chloro-1-phenylpropyl]oxy}-1,2-dimethoxybenzene (53.4 mg, 0.174 mmol) in DMF (0.5 mL) was stirred at 100 °C for 3 hrs, at which time TLC indicated that the reaction was complete. The reaction mixture was poured into water (5.0 mL) and the aqueous layer was extracted with methylene chloride (3x30 mL). The combined organic extracts were washed with brine (30 mL), dried over MgSO₄ and concentrated under reduced pressure. The crude product was purified by Prep-TLC plates [1:1=hexane:ethyl acetate with 1% NH₃] afforded the product (63.1 mg, 76.6 %) as a yellow oil. The product was used in next reaction without further purification.

Step 2:

4-{1-[*(3R)*-3-(3,4-DIMETHOXYPHENOXY)-3-PHENYLPROPYL]-4-PIPERIDINYL}ANILINE: A 25-mL RB flask, equipped with a hydrogen-filled balloon, was charged with 1-[*(3R)*-3-(3,4-dimethoxyphenoxy)-3-phenylpropyl]-4-(4-nitrophenyl)-1,2,3,6-tetrahydropyridine (63.0 mg, 0.133 mmol), Palladium on Carbon (5.0 mol-eq%, 0.00665 mmol, 7.04 mg) and ethanol (2.0 mL) at room temperature. After 1 hr the reaction mixture was filtered through a plug of Celite 545 and concentrated under reduced pressure. The crude product (54.1 mg, 89.4%) was used in next reaction without further purification.

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STEP 3:

N-(4-{1-[*(3R)*-3-(3,4-DIMETHOXYPHENOXY)-3-PHENYLPROPYL]-4-PIPERIDINYL}PHENYL)-2-METHYLPROPANAMIDE: A mixture of 4-{1-[*(3R)*-3-(3,4-dimethoxyphenoxy)-3-phenylpropyl]-4-piperidinyl}aniline (5.31 mg, 0.0119 mmol), isobutyryl chloride (2.08 mg, 0.019 mmol), *N,N*-diisopropylethylamine (8.40 mg, 0.0650 mmol) in methylene chloride (1.0 mL) was stirred at room temperature for 24 hours. The reaction mixture was concentrated and chromatographed using a preparative TLC plate [2.5% of NH₃ (2.0 M in methanol) in CHCl₃] to give the product (3.5 mg, 56.5 %) as a thick oil: ¹H NMR (400 MHz, CDCl₃) δ 7.38 (d, 1H, J=8.6 Hz), 7.30-7.20 (m, 4H), 7.20 (m, 1H), 7.11 (d, 2H, J=8.6 Hz), 7.04 (s, 1H), 6.57 (d, 1H, J=8.3 Hz), 6.44 (d, 1H, J=2.6 Hz), 6.22 (dd, 1H, J=2.6, 8.3 Hz), 5.09 (apparent dd, 1H, J=4.4, 8.1 Hz), 3.72 (s, 3H), 3.70 (s, 3H), 3.08 (m, 2H), 2.57 (m, 2 H), 2.43 (apparent sept, partially hidden, 2H, J=6.8 Hz), 2.30-2.10 (m, 6H), 1.80 (m, 2H), 1.25 (d, 6H, J=7.9 Hz); ESMS m/e: 517.3 (M+H)⁺.

Example 141

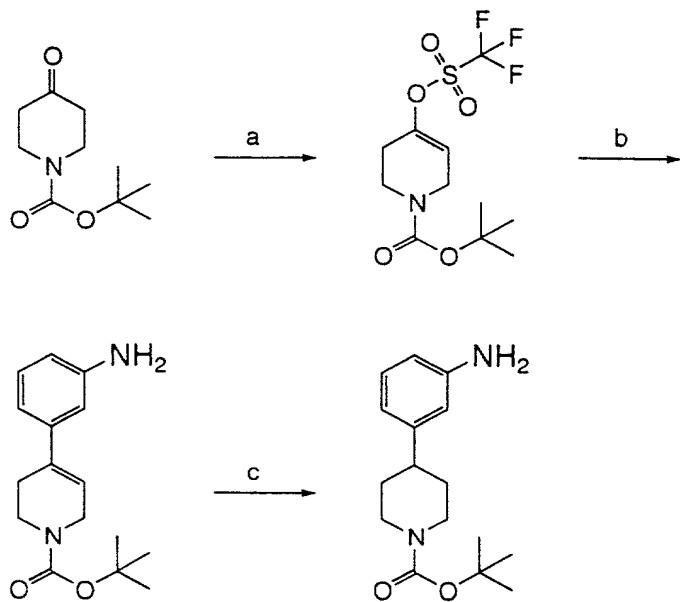
N-(3-{1-[(3S)-3-(3-ACETYLPHENOXY)-3-PHENYLPROPYL]-4-
5 PIPERIDINYLPHENYL}-2-METHYLPROPANAMIDE

Into a 25-mL RB-flask was added triphenylphosphine (9.80 mg, 0.0375 mmol), diethyl azodicarboxylate (5.22 mg, 0.0300 mmol), N-(3-{1-[(3R)-3-hydroxy-3-phenylpropyl]-4-
10 piperidinyl}phenyl)-2-methylpropanamide (9.53 mg, 0.0250 mmol), 3-hydroxyacetophenone (100 mg) and THF (1.0 mL) at room temperature. The reaction mixture was stirred at room temperature overnight (16 hrs). The solvent was removed under reduced pressure and the residue was purified by preparative TLC plates [2.5% of NH₃ (2.0 M in methanol) in CHCl₃] to afford the desired product (2.73 mg, 39.9%) as a thick oil: ¹H NMR δ 7.70-7.64 (m, 2H), 7.54 (m, 2H), 7.49-7.44 (m, 6H), 7.25 (m, 1H), 7.05 (d, 1H, J=8.3 Hz), 6.96 (apparent d, 1H, J=7.7 Hz), 5.34 (apparent dd, 1H, J=4.8, 8.2 Hz), 3.15 (m, 2H), 2.67 (m, 2H), 2.52 (s, 3H), 2.53 (apparent sept, partially hidden, 2H, J=7.6 Hz), 2.30-2.10 (m, 6H), 1.89 (m, 2H), 1.25 (d, 6H, J=6.9 Hz); ESMS m/e: 499.4 (M + H)⁺.

25

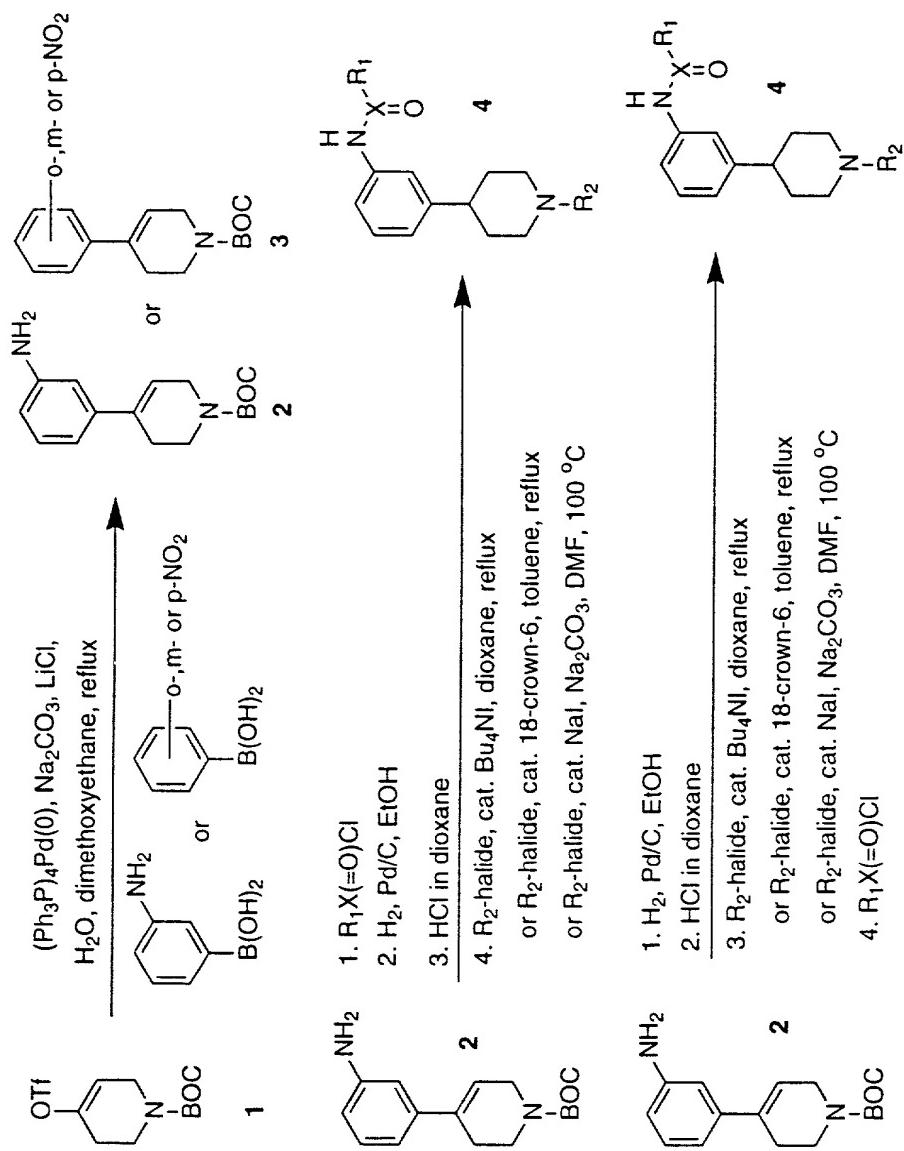
30

Scheme A. Synthesis of tert-Butyl 4-(3-aminophenyl)-1-piperidinecarboxylate

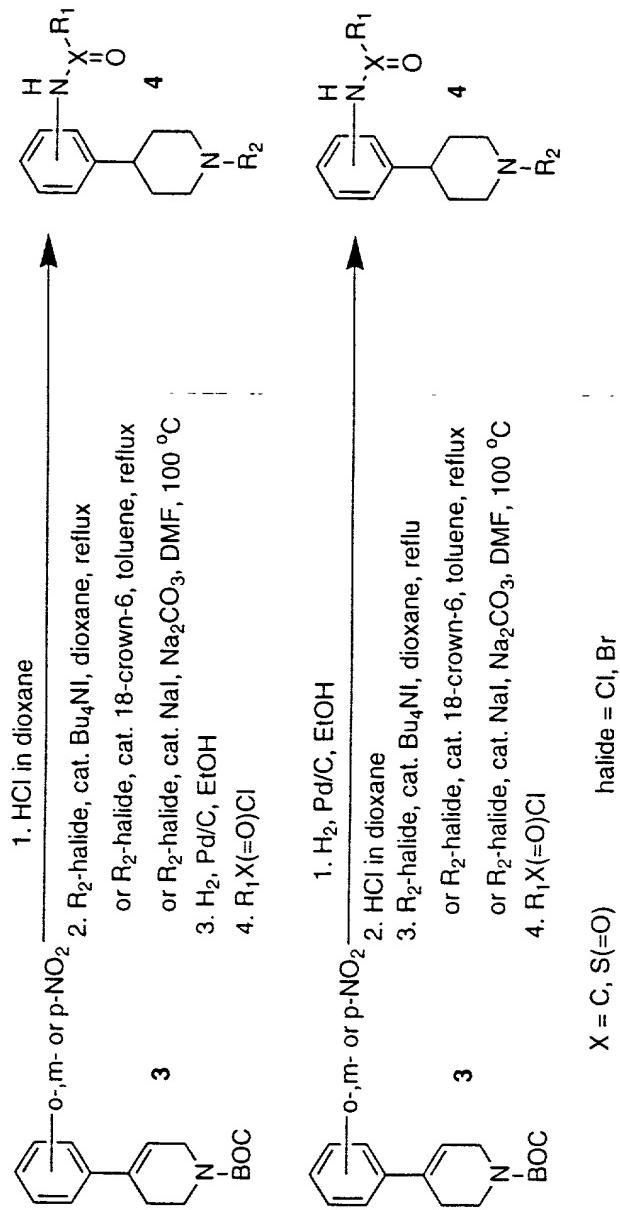


- a. n-BuLi, diisopropylamine, THF, PhN(Tf)₂, -78 °C to room temperature, 81%
- b. 3-aminophenylboronic acid hemisulfate, LiCl, tetrakis-triphenylphosphine-palladium (0), Na₂CO₃, DME-H₂O, reflux, 81%
- c. 10% Pd/C, ethanol, H₂, room temperature, balloon method, 84%

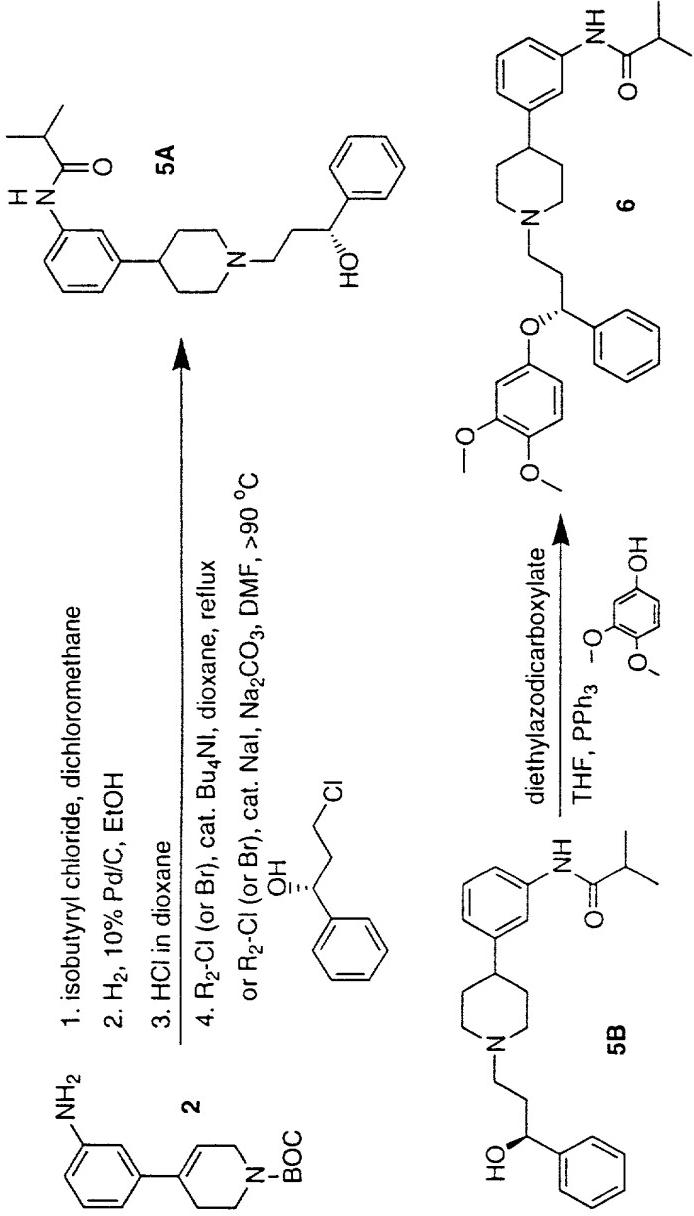
Scheme B1. A General Synthesis of the MCH Antagonists

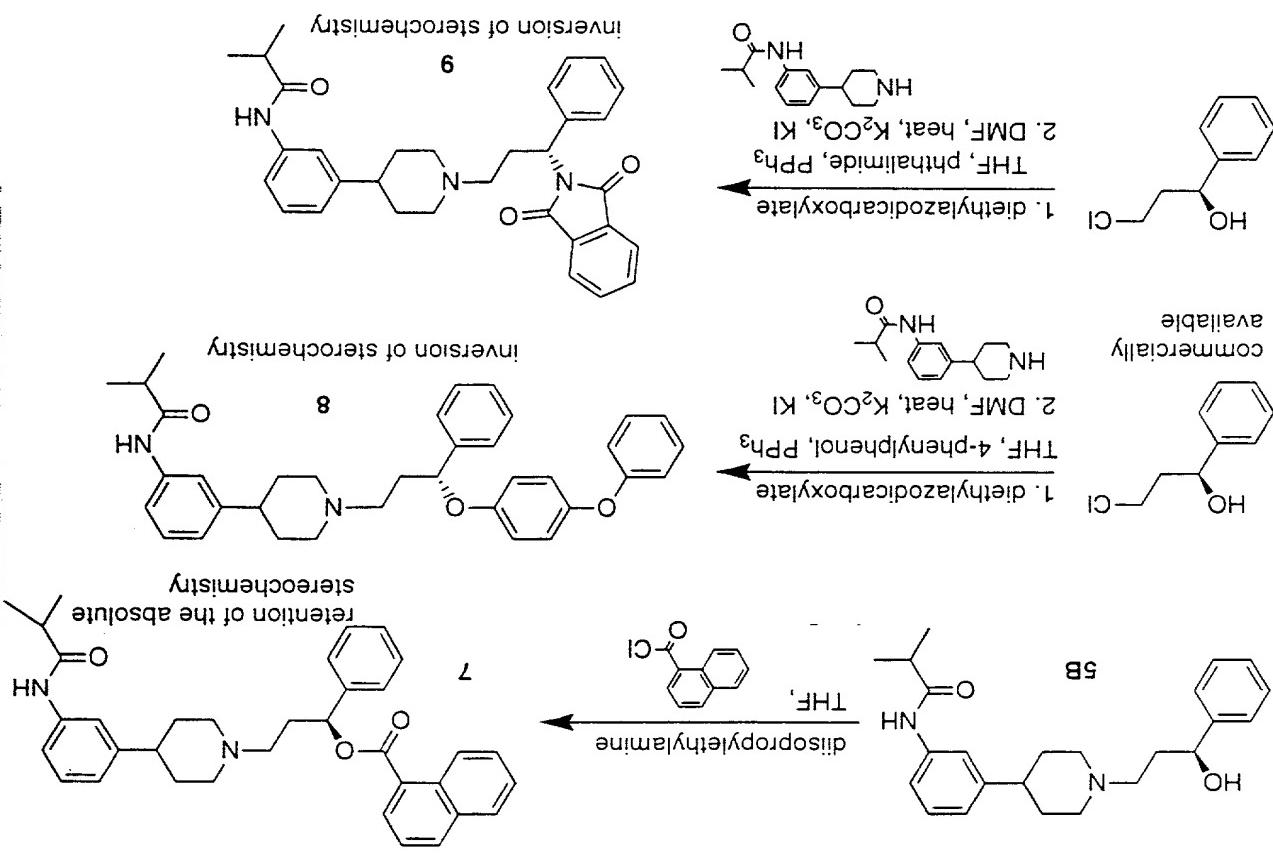


Scheme B2. A General Synthesis of the MCH Antagonists



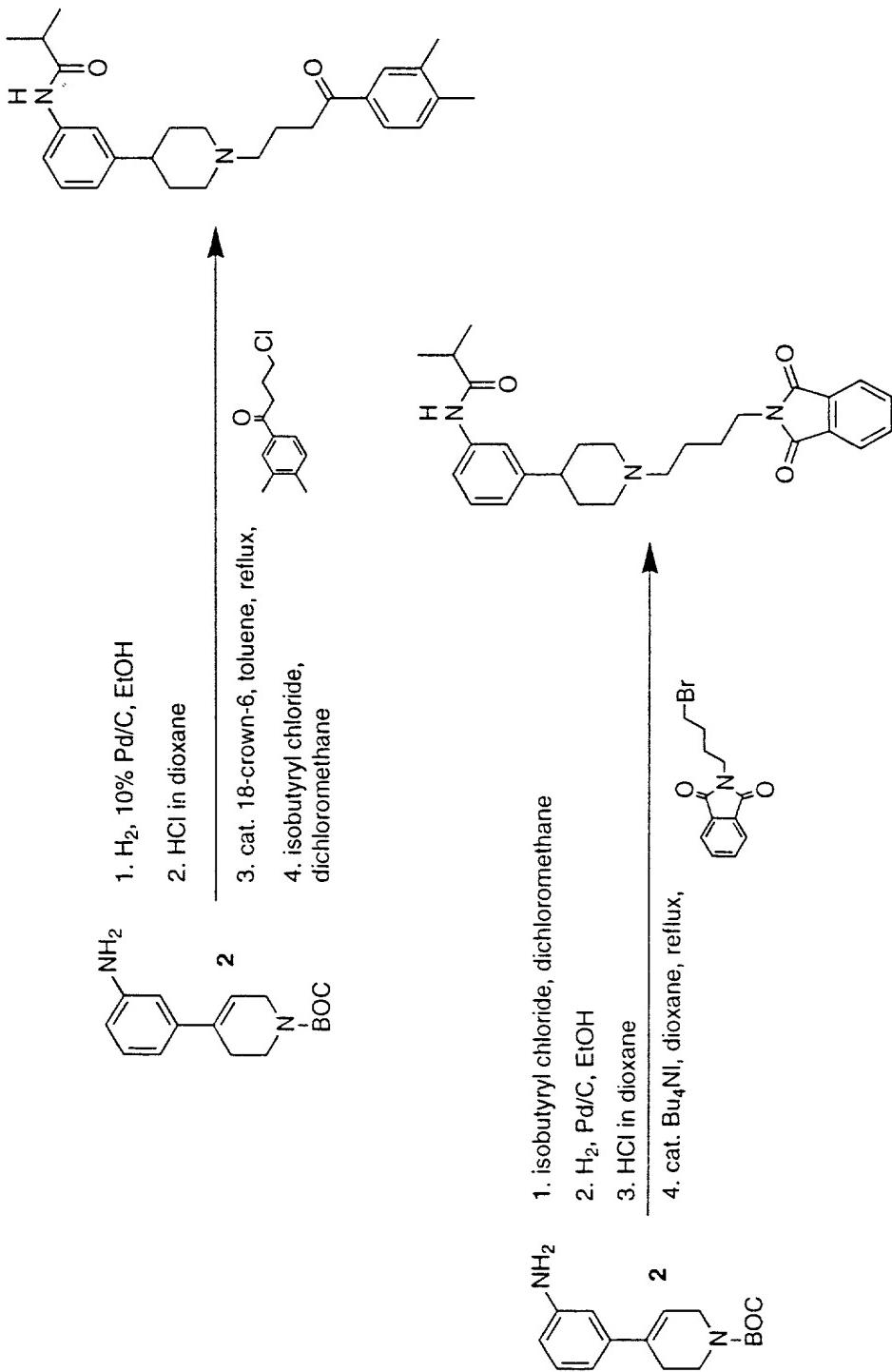
Scheme C1. Specific Examples of the Syntheses of the MCH Antagonists



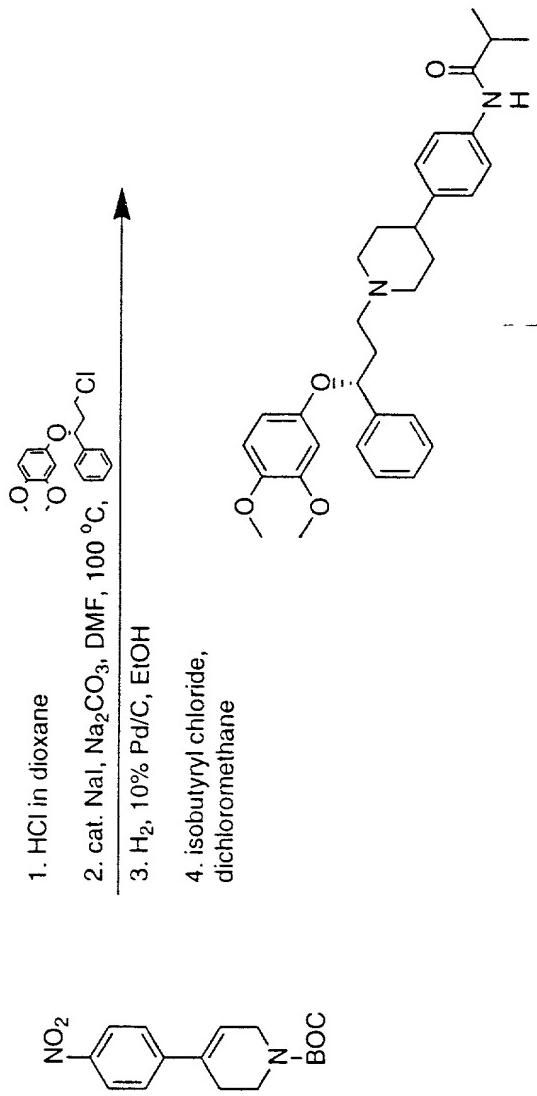


Scheme C2. Specific Examples of the Syntheses of the MCH Antagonists

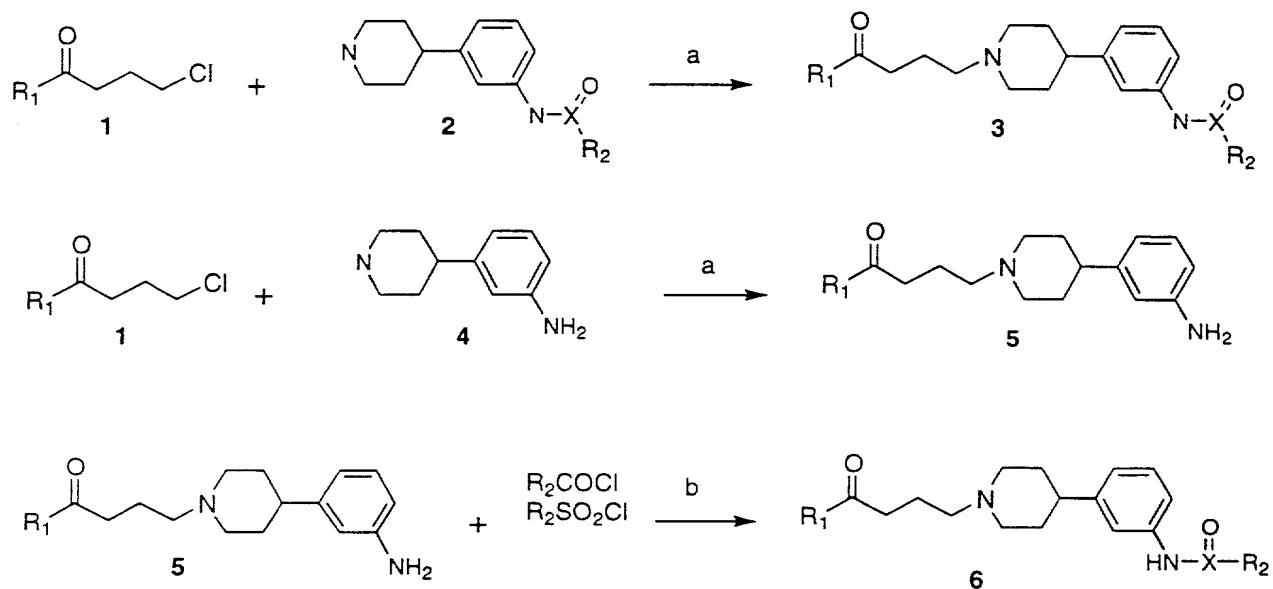
Scheme D1. Specific Examples of the Syntheses of the MCH Antagonists



Scheme D2. Specific Examples of the Syntheses of the MCH Antagonists



Scheme E: General Synthesis of the MCH Antagonists



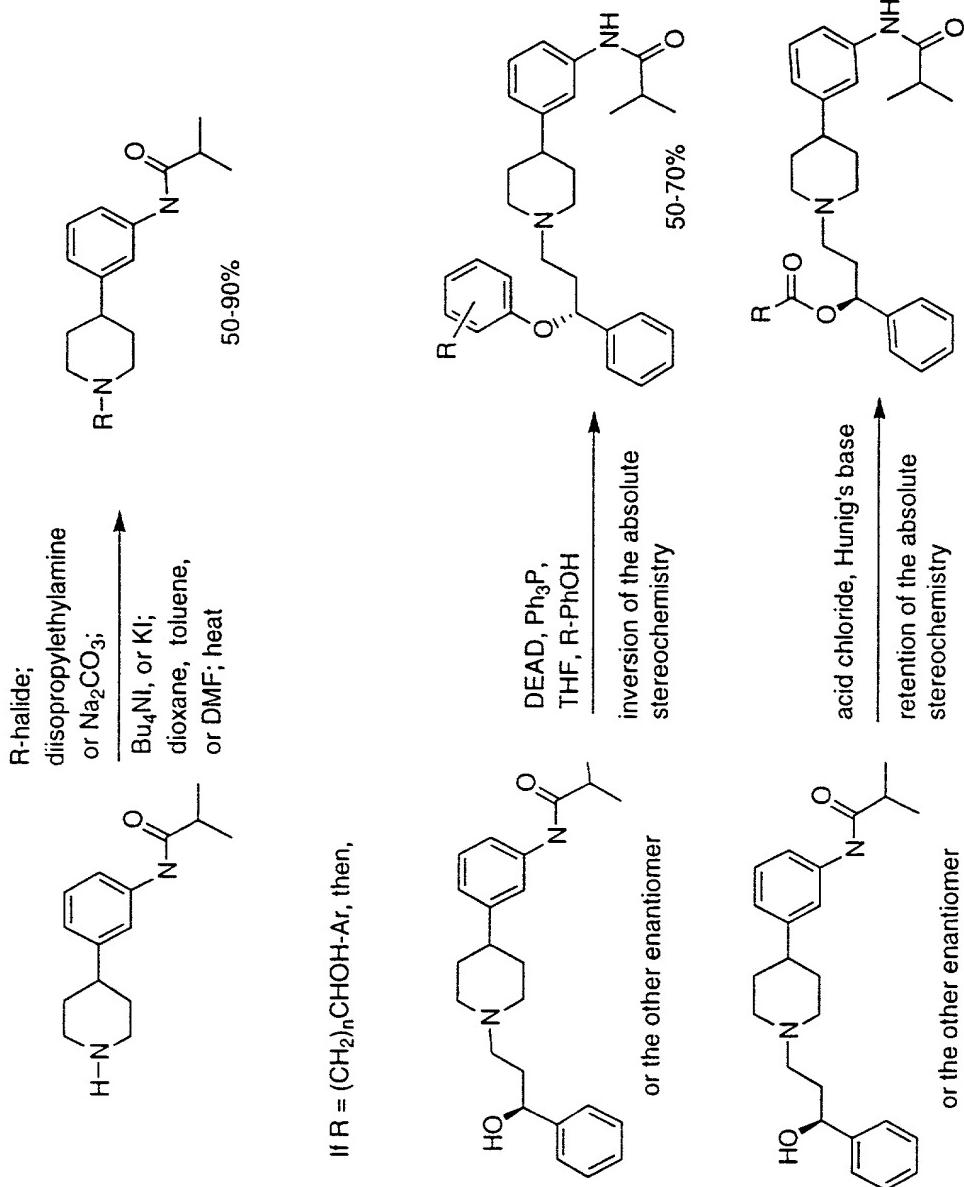
a. dioxane, diisopropylethylamine, Bu₄NI, reflux
or DMF, K_i, Na₂CO₃, 90-100 °C
or toluene, 110 °C, 18-crown-6
b. diisopropylethylamine, dichloromethane

X = S(=O), C

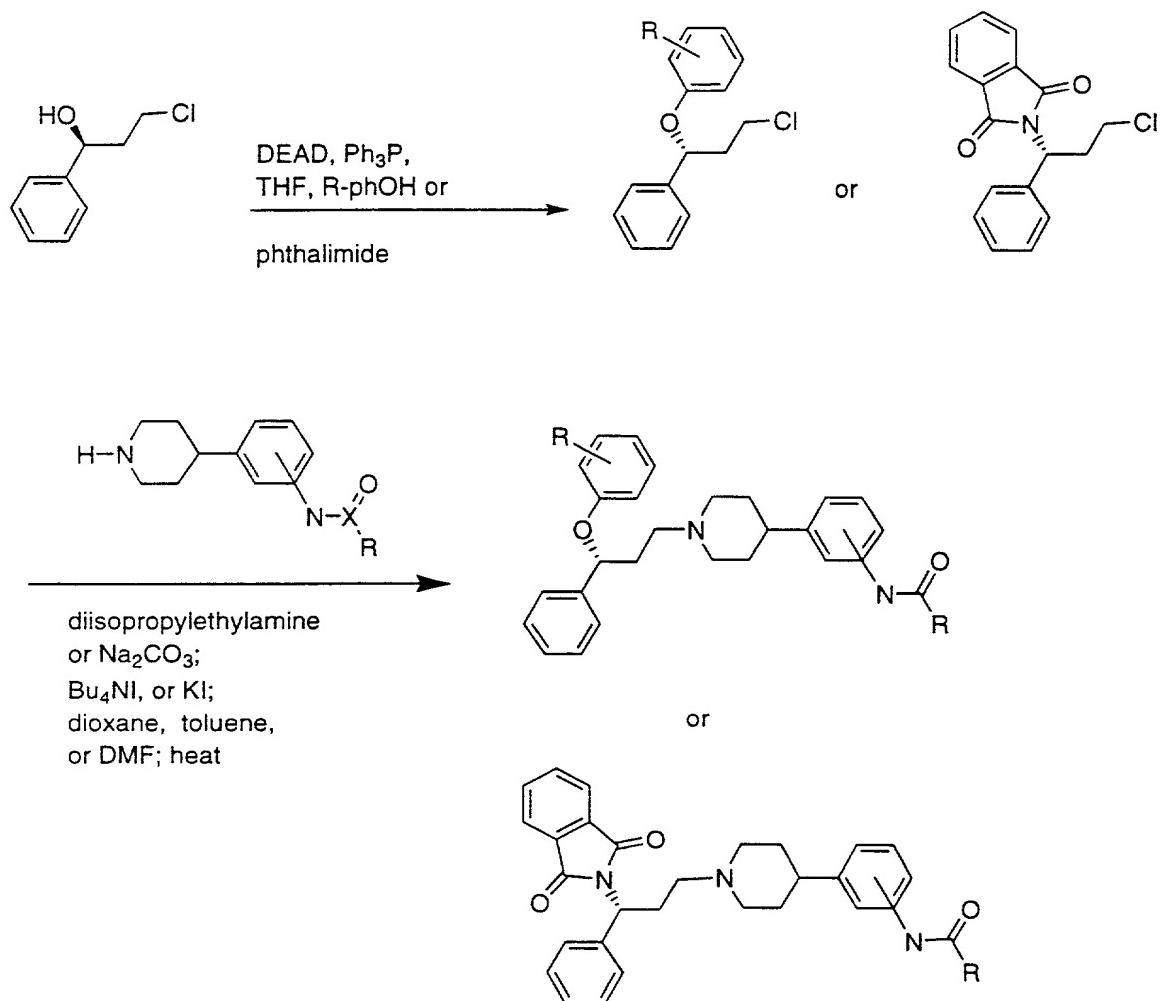
R₁ = Aromatic, substituted aromatic or heterocyclic

R₂ = aliphatic or aromatic

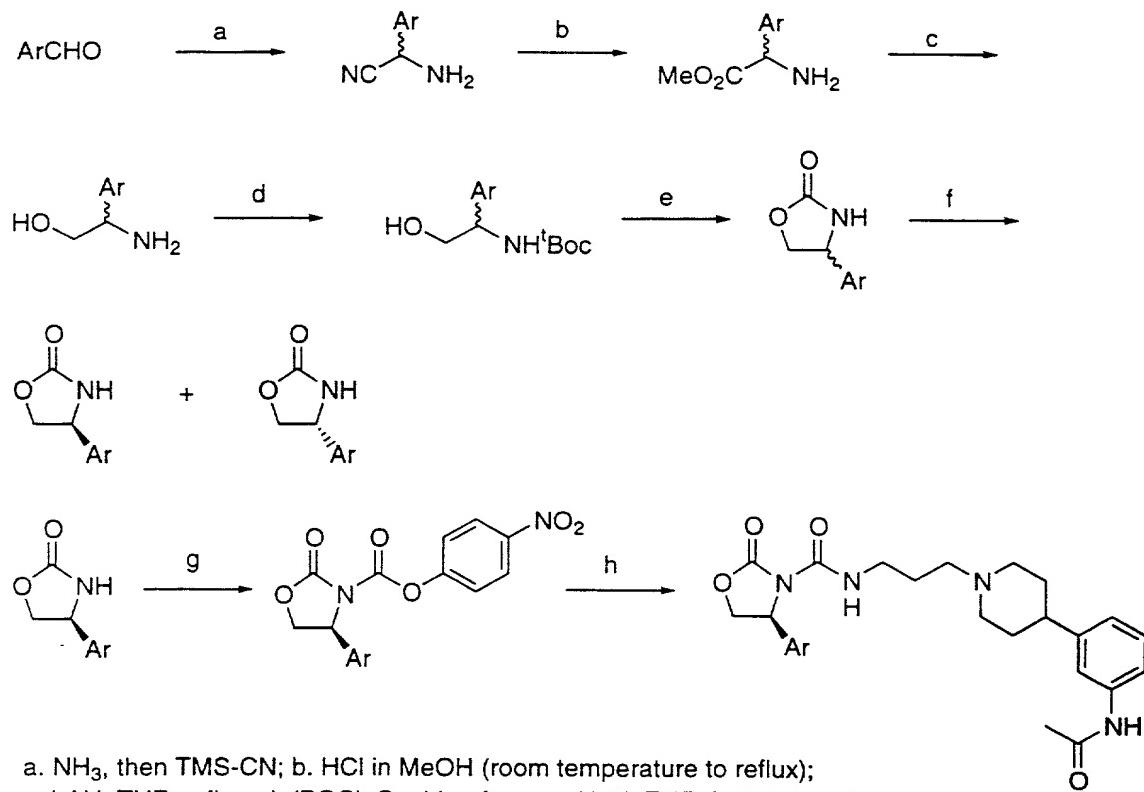
Scheme F. General Synthesis of the MCH Antagonists



Scheme G. General Synthesis of the MCH Antagonists



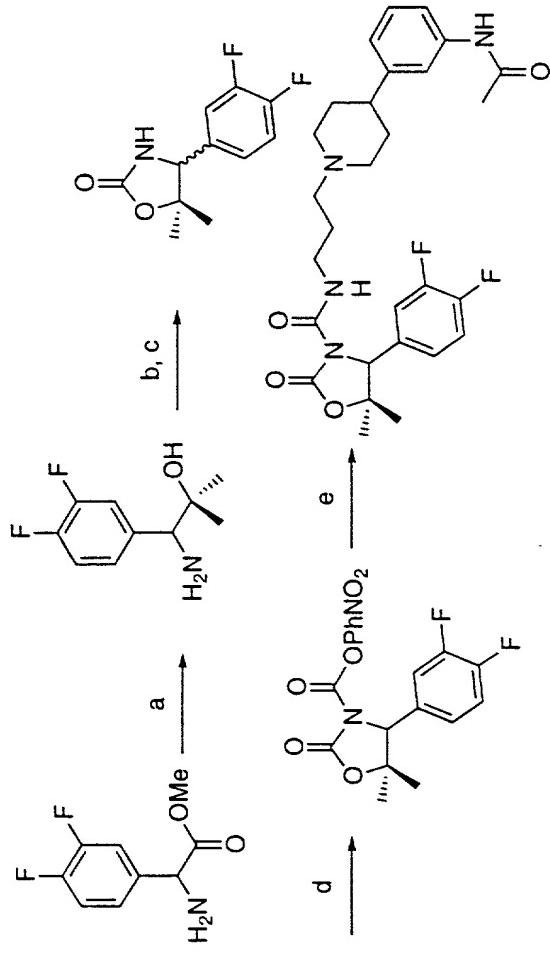
Scheme H: Synthesis of Oxazolidinones



- a. NH_3 , then TMS-CN; b. HCl in MeOH (room temperature to reflux);
c. LAH, THF, reflux; d. $(\text{BOC})_2\text{O}$, chloroform; e. NaH, THF; f. Chiralcel OD column
g. NaH, p-nitrophenyl chloroformate, THF;
h. an amine such as N-(3-[1-(3-aminopropyl)-4-piperidinyl]phenyl)acetamide

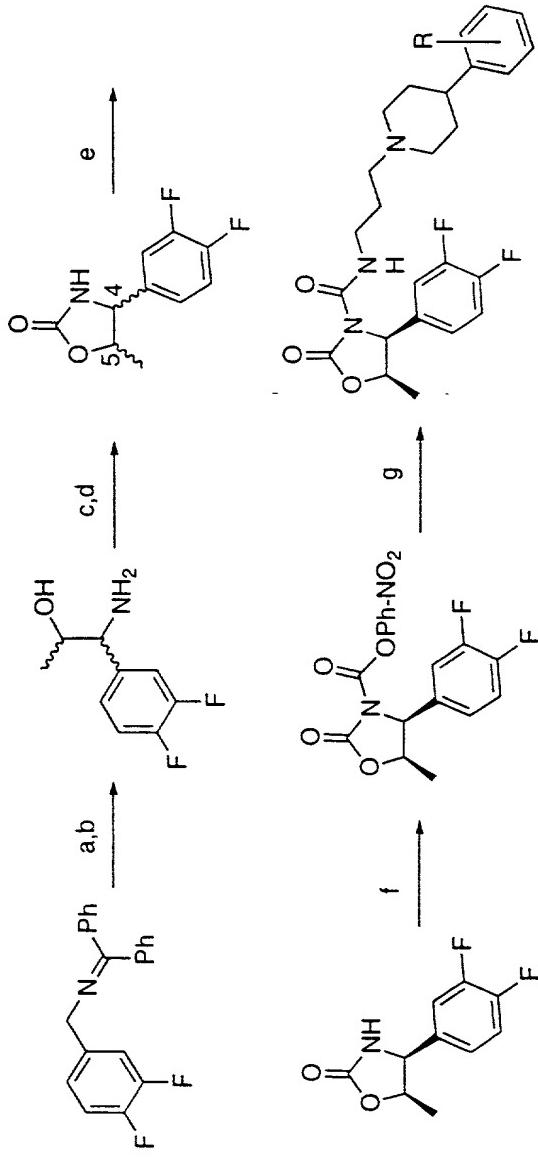
Ar = 3,4-difluorophenyl, 3,5-difluorophenyl or 3,4,5-trifluorophenyl

Scheme I: Synthesis of gem-Dialkyl Substituted Oxazolidinones



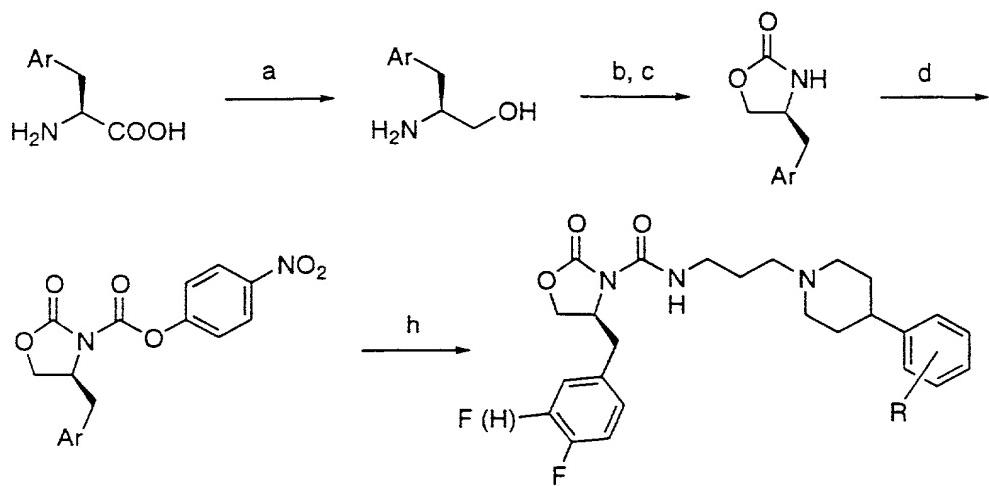
a. methyl magnesium bromide, THF; b. $(BOC)_2O$, chloroform; c. NaH, THF,
p-nitrophenylchloroformate; e. an amine such as
N-(3-[1-(3-aminopropyl)-4-piperidinyl]phenyl)acetamide

Scheme J: Synthesis and Chiral Resolution of Oxazolidinones



a (a) *t*-BuLi, THF, RCHO (b) $\text{CH}_3\text{ONH}_2 \cdot \text{HCl}$, MeOH, 50-68% over 2 steps (c) Boc_2O , CHCl_3 , >90% (d) NaH , THF, 76-92% (e) separate diastereomers by column chromatography and separate enantiomers by chiral phase HPLC, 10-16% (f) NaH , THF, 4-nitrophenylchloroformate, ~75%
(g) THF, >80%, an amine such as N-[3-{1-(3-aminopropyl)-4-piperidinyl}phenyl]acetamide

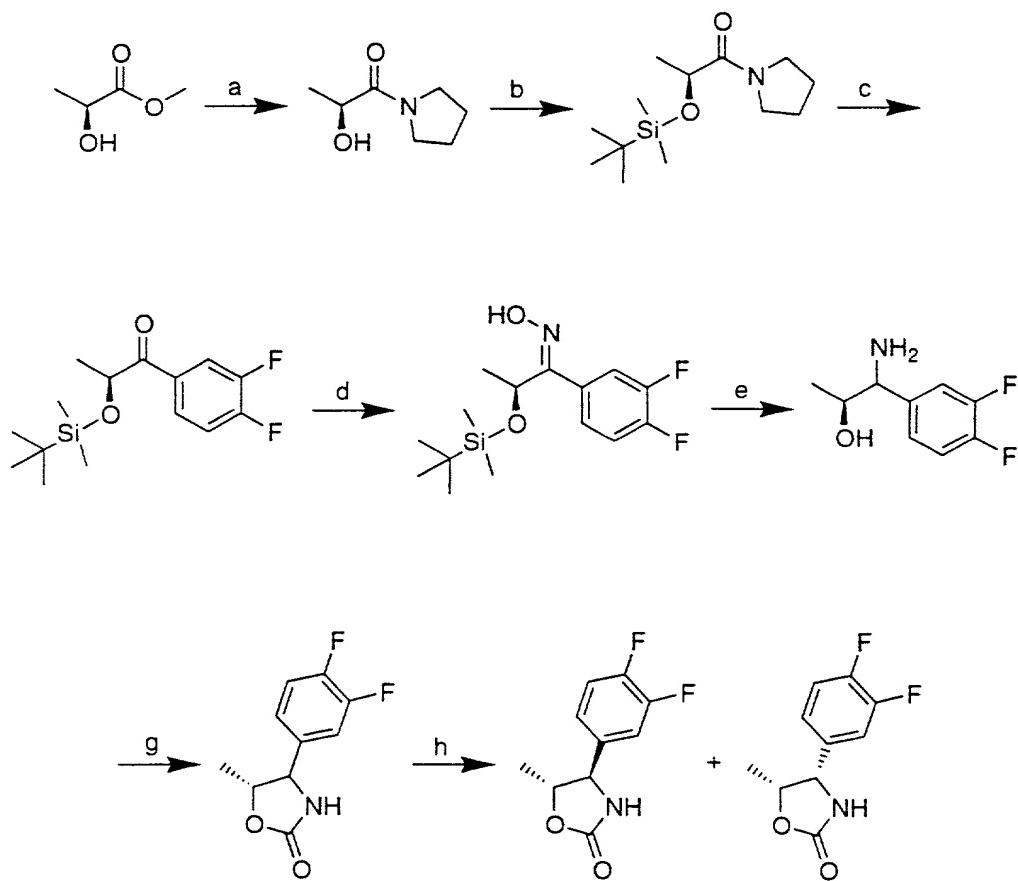
Scheme K: Synthesis Oxazolidinones from Amino Acids



a. LAH, THF; b. $(\text{BOC})_2\text{O}$, CHCl_3 ; c. NaH , THF; d. p -nitrophenylchloroformate, NaH , THF;
h. an amine such as $\text{N}\{-3\text{-[1-(3-aminopropyl)-4-piperidinyl]phenyl}\}\text{acetamide}$

Ar = aromatic such as 4-fluorophenyl or 3,4-difluorophenyl

Scheme L: Determination of the Absolute Stereochemistry of the Di-Substituted Oxazolidinones Using Lactic Acid Derivatives



- a. pyrrolidine, methanol, heat; b. t-butyldimethylsilyl chloride; c. LAH, ether, reflux
d. $(\text{BOC})_2\text{O}$, chloroform; e. NaH, THF; h. silica gel chromatography

For more details, See: Lagu, B.; Wetzel, J. M.; Forray, C.; Patane, M. A.; Bock, M. G.
"Determination of the Relative and Absolute Stereochemistry of a Potent $\alpha 1\text{A}$ Selective Adrenoceptor Antagonist" Bioorg. Med. Chem. Lett. 2000, 10, 2705.

Table 1 (Continued)

EXAMPLE No.	STRUCTURE	K _i (nM) rMCH1
38		1.34
39		3.33
40		2.72
41		0.04
42		0.6
43		0.23
44		0.09

Table 1

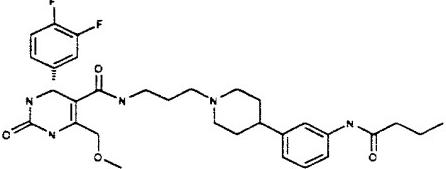
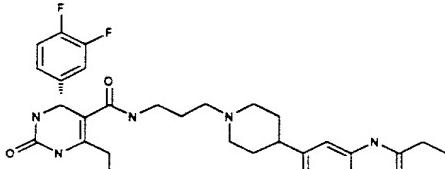
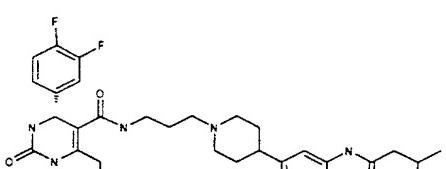
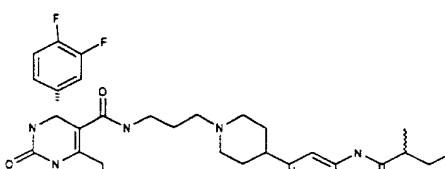
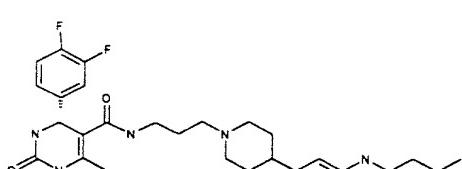
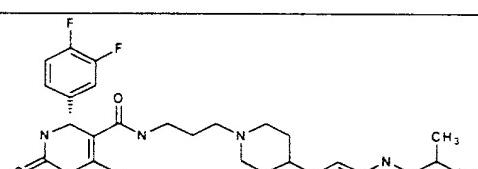
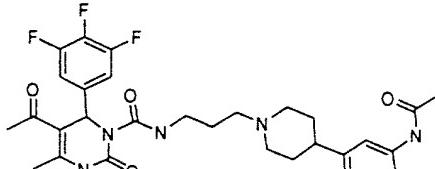
45		14.69
46		8.16
47		34.28
48		22.15
49		225.47
50		13.74
51		0.79

Table 1

52		0.81
53		50.76
54		29.87
55		203.74
56		0.26
57		90
58		3.9
59		768

Table 1

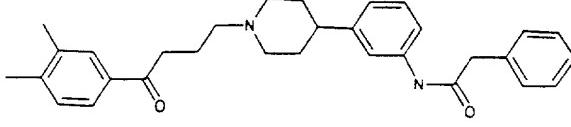
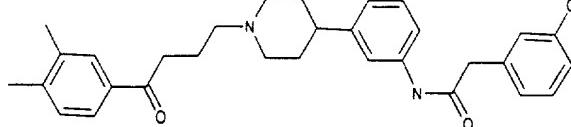
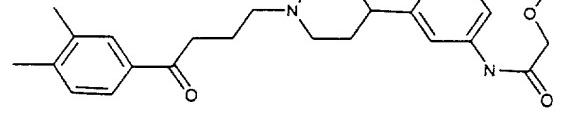
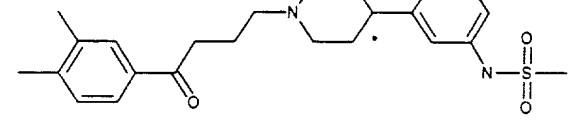
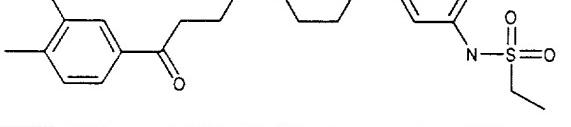
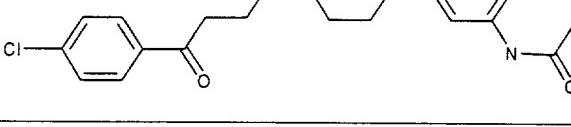
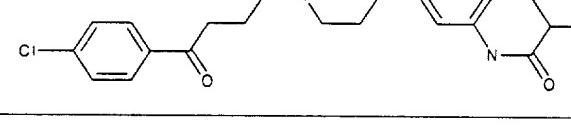
60		357
61		14.2
62		274
63		1000
64		627
65		69
66		2.8
67		197
68		84

Table 1

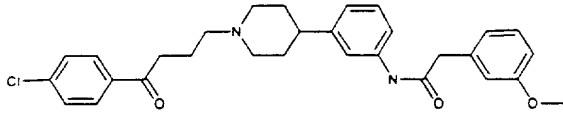
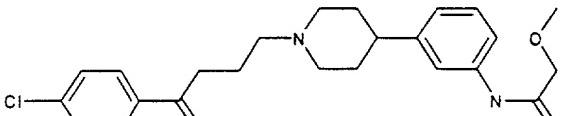
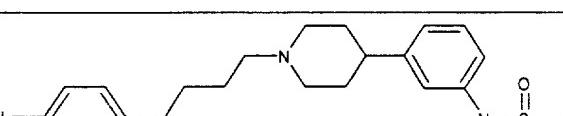
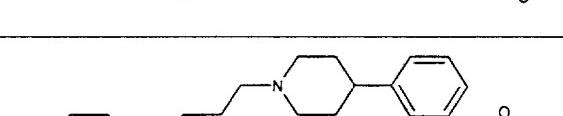
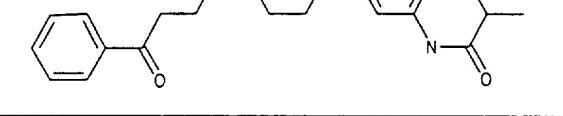
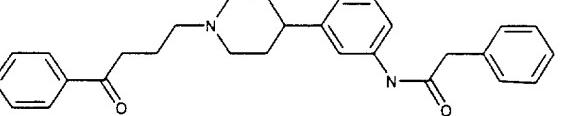
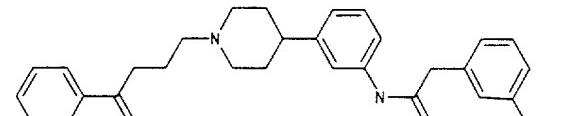
69		11.9
70		167
71		720
72		272
73		342
74		29.5
75		506
76		21
77		630

Table 1

78		52
79		1036
80		67
81		463
82		192
83		91
84		511
85		654
86		382

Table 1

87		362
88		160
89		615
90		651
91		11.5
92		62
93		29.1
94		18.2
95		11.8

Table 1

96		50
97		946
98		118
99		12
100		11.5
101		1.6
102		187
103		52
104		6.7

Table 1

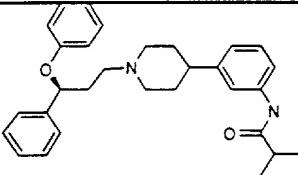
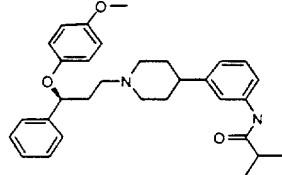
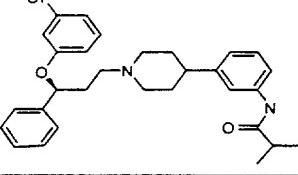
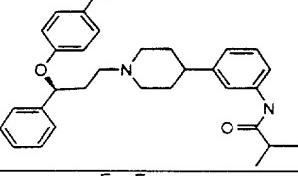
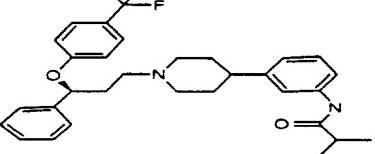
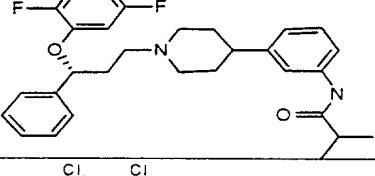
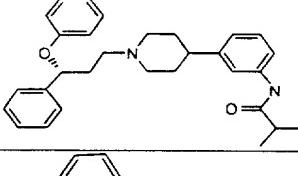
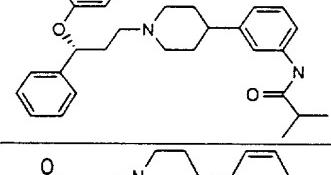
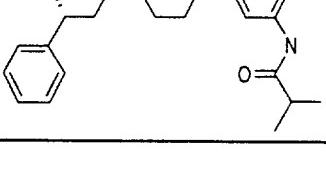
105		7.1
106		3.9
107		3.1
108		3.8
109		7.1
110		4.9
111		5
112		22.3
113		16.6

Table 1

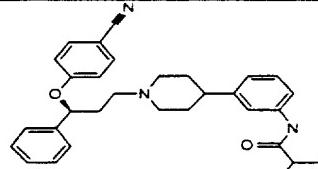
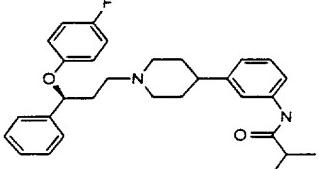
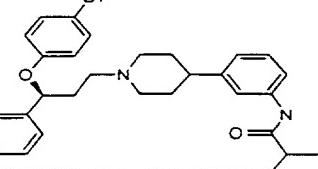
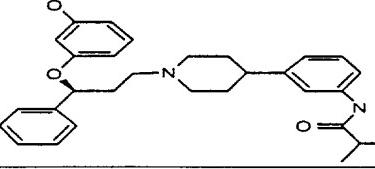
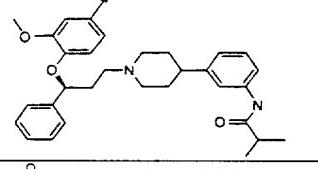
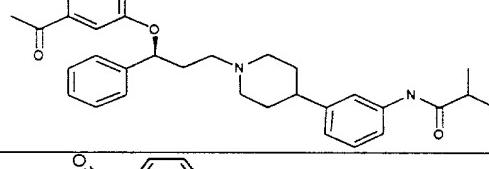
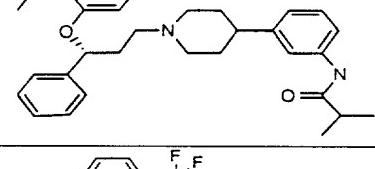
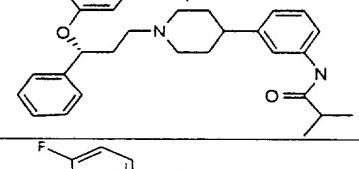
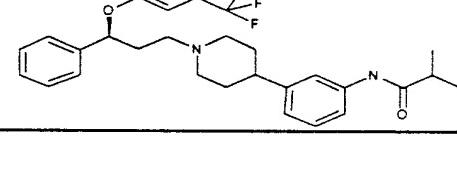
114		2.01
115		12.9
116		0.923
117		13.6
118		12.8
119		22.4
120		14.8
121		17
122		3.3

Table 1

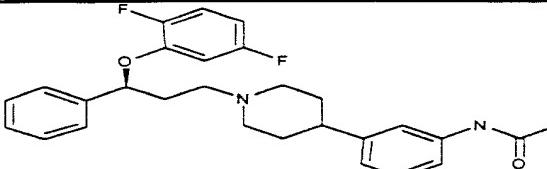
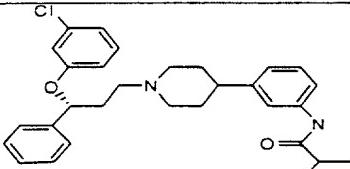
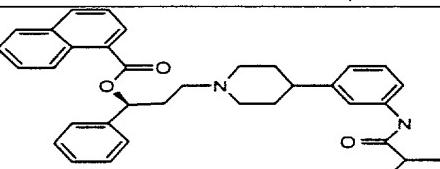
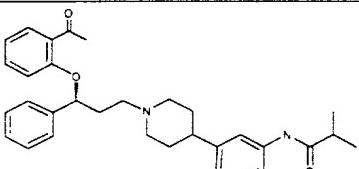
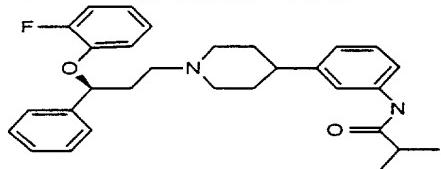
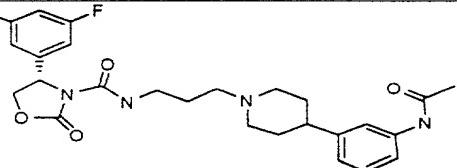
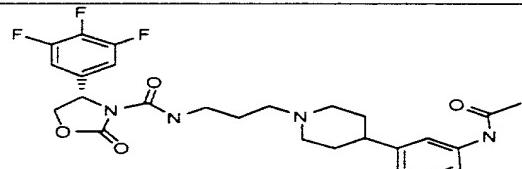
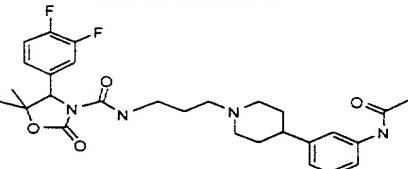
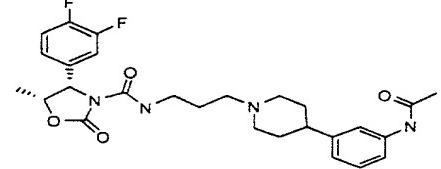
123		5.9
124		9.3
125		32.5
126		50
127		6.6
128		31.4
129		22.3
130		48.6
131		11.8

Table 1

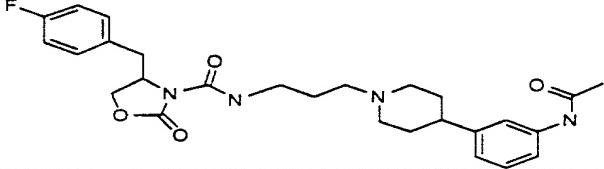
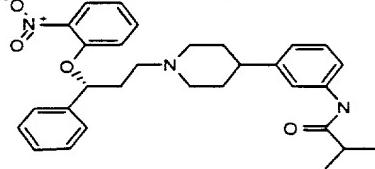
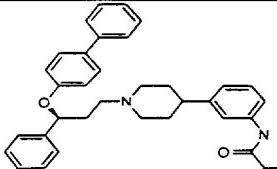
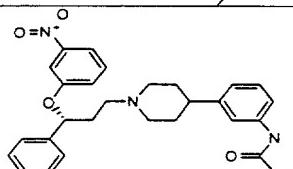
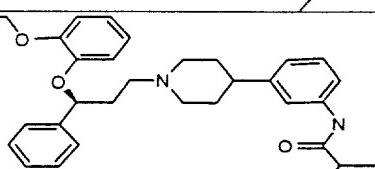
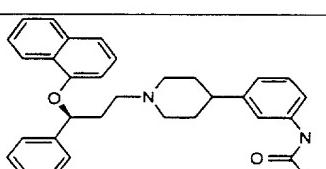
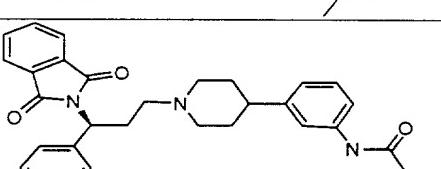
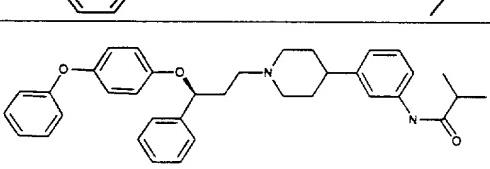
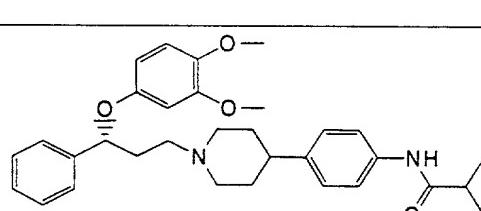
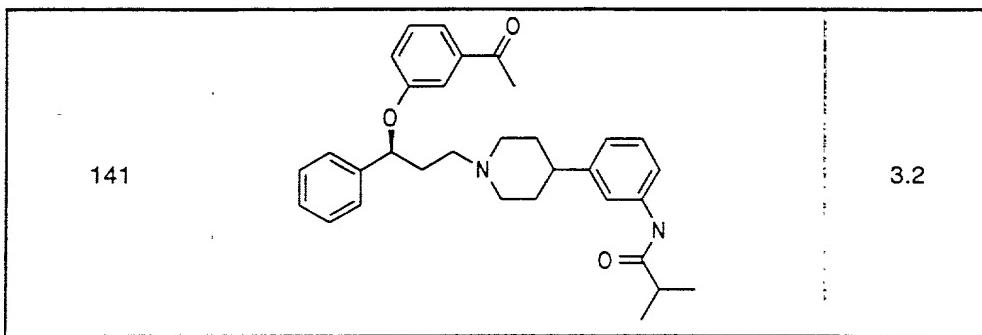
132		44.6
133		25.7
134		22.2
135		19.4
136		14.3
137		377
138		11.2
139		48.1
140		121

Table 1



In Vivo Models

Materials and Methods

5 1. Effects on MCH-Stimulated Food Intake

To determine if an MCH1 antagonist could attenuate MCH-stimulated food intake, the effect of an i.p. dose of Compound 10 on food intake induced by intracerebral ventricularly administered MCH was measured.

10 Animals

Adult male albino Wistar rats (Charles River Laboratories, NY) were housed individually and maintained on a 12h light dark cycle and given free access to Purina rat chow and water. Rats were pretreated with chlorpromazine (3 mg/kg, i.p.) and anesthetized with Ketamine HCl (120 mg/kg, i.m.). A stainless steel cannula (22 gauge, Plastics One, Roanoke, VA) was implanted stereotactically (Kopf Instrumtns, Tujunda, CA) aimed at the third ventricle using the following coordinates: incisor bar (+5 mm), 3.0 mm posterior to Bregma, 1.5 mm lateral and angled 10° towards the sagittal suture, and 9 mm from the top of the skull. The cannula was secured to the skull by 4 anchor screws with dental acrylic. Animals were allowed 10 days to recover before testing began.

25 Testing Paradigm

Rats were habituated to the testing paradigm over several days in which the food bin was removed from the home cage, and preweighed food pellets were placed on the floor of the animal's cage at 3-6 hours into the light cycle. Animals were considered to have met a baseline criterion of minimal food intake (<1 g over 2 hours) after 2 consecutive days. Rats were then administered vehicle (artificial CSF, 5 ul, 1 ul/15 sec) into the third

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ventricle via a stainless steel internal cannula (28-gauge, Plastics One) connected to a Hamilton microsyringe by polyethylene tubing. Food was introduced on the floor of the cage immediately after injection and intake was assessed 30, 60 and 120 min after. After verifying low levels of intake following vehicle administration, MCH (3 nmol, 5 ul) was microinjected into the third ventricle and food intake assessed as above. Subgroups of these rats were then tested with the following pairs of injections in counterbalanced order with a minimum of 4 days elapsing between injection conditions: a) DMSO (1%, i.p.) 10 min prior to MCH (third ventricle, 3 nmol, 5 ul, n=11), b) Compound 10 (1 mg/kg, i.p.) 10 min before MCH (third ventricle, 3 nmol, 5 ul, n=8), and c) Compound 10 (10 mg/kg, i.p.) 10 min before MCH (third ventricle, 3 nmol, 5 ul, n=6). Food was introduced immediately after the second injection and intake assessed as above.

2. Effects of MCH1 Antagonists on Body Weight

Male Long Evans rats (Charles River) weighing 180-200 grams at the start of experiments were housed in pairs (osmotic minipump experiment) or groups of four (i.p. injections) on a 12 hour light/dark cycle with free access to food and water.

For studies involving osmotic minipumps, rats were anesthetized with isoflurane (Aerrane, Baxter Pharmaceutical) and an osmotic minipump (model 2ML2, Alzet, Palo Alto, CA) filled with either vehicle (20 % DMSO), Compound 10 (19.2 mg/ml in 20 % DMSO) or d-fenfluramine (Sigma, St. Louis MO; 11.5 mg/ml in 20% DMSO) was implanted subcutaneously into the mid scalpular region. At these concentrations, rats received continuous infusions of 10 mg/kg/day of Compound 10 or 6 mg/kg/day of d-fenfluramine.

For studies involving i.p. injections, drugs were administered twice daily, once 1 hour before the dark cycle and once 2 hours after lights on. All rats were weighed daily after the morning injection. Overall results were analyzed by two-way ANOVA, data for each time point were analyzed by one-way ANOVA followed by post hoc Student-Newman-Keuls test.

3. Effects of MCH1 Antagonists on Consumption of Sweetened Condensed Milk

Male Sprague Dawley rats (Charles River) weighing 180-200 grams at the start of experiments were housed in groups of four on a 12 hour light/dark cycle with free access to food and water. For 7 days, rats were weighed, placed in individual cages and allowed to drink sweetened condensed milk (Nestle, diluted 1:3 with water) for 20 min 2-5 hours into the light cycle. The amount of milk consumed was determined by weighing the milk bottle before and after each drinking bout. On the test day, rats received i.p. injections of Compound 10 (3, 10 or 30 mg/kg in 0.01 % lactic acid), vehicle (0.01 % lactic acid) or d-fenfluramine (3 mg/kg in 0.01 % lactic acid) 30 min prior to exposure to milk. The amount of milk consumed on the test day (in mls milk/ kg body weight) was compared to the baseline consumption for each rat determined on the previous 3 days. Data was analyzed using a two-tailed unpaired t-test.

4. Forced Swim Test (FST)

The procedure used in this study was similar to that previously described (Porsolt, et al., 1978), except the water depth (30 cm in this procedure). The greater depth in this test prevented the rats from supporting themselves by touching the bottom of the cylinder with their feet. Swim sessions were conducted by placing rats in individual

plexiglass cylinders (46 cm tall x 20 cm in diameter) containing 23-25°C water 30 cm deep (Porsolt, et al. used a depth of only 15 cm; also, see Detke, et al., 1995). Two swim tests were conducted always between 1200 and 1800 hours: an initial 15-min pretest followed 24 h later by a 5-minute test. Drug treatments were administered 30 minutes before the 5-minute test period. All other test sessions were conducted between 1300 to 1700 hours. Following all swim sessions, rats were removed from the cylinders, dried with paper towels and placed in a heated cage for 15 minutes and returned to their home cages. All test sessions were videotaped using a Panasonic color video camera and recorder for scoring later.

15 Animals

Male Sprague-Dawley rats (Taconic Farms, NY) were used in all experiments. Rats were housed in pairs and maintained on a 12:12-h light-dark cycle. Rats were handled for 5 minutes each day for 5 days prior to behavioral testing.

20

Behavioral Scoring

The rat's behavior was rated at 5 second intervals during the 5 minute test as one of the following:

25

1. Immobility- rat remained floating in the water without struggling and was only making those movements necessary to keep its head above water;

30

2. Climbing - rat was making active movements with its forepaws in and out of the water, usually directed against the walls;

35

3. Swimming - rat was making active swimming motions, more than necessary to merely maintain its head

above water, e.g. moving around in the cylinder; and

4. Diving - entire body of the rat was submerged.

5 All of the behavior scoring was done by a single rater, who was blind to the treatment condition.

Drug Administration

10 Animals were randomly assigned to receive a single i.p. administration of Compound 10 (3, 10 or 30 mg/kg, dissolved in 5% lactic acid), fluoxetine (10 mg/kg, dissolved in distilled water) or vehicle (equal mixture of 5% lactic acid and distilled water) 30 minutes before the start of the 5 minute test period. All injections were
15 given using 1 cc tuberculin syringe with 26 3/8 gauge needles (Becton-Dickinson, VWR Scientific, Bridgeport, NJ). The volume of injection was 1 ml/kg.

20 The effect of 10 mg/kg of fluoxetine was utilized in the FST as a positive control.

Data Analysis

25 The forced swim test data (immobility, swimming, climbing, diving) were subjected to a randomized, one-way ANOVA and post hoc tests conducted using the Student-Newman-Keuls test. The data were analyzed using the GBSTAT program, version 6.5 (Dynamics Microsystems, Inc., Silver Spring, MD, 1997). All data are presented as means \pm S.E.M.

30 5. Social Interaction Test (SIT)

Rats were allowed to acclimate to the animal care facility for 5 days and were housed singly for 5 days prior to testing. Animals were handled for 5 minutes per day. The design and procedure for the Social Interaction Test was carried out as previously described by Kennett, et al.

(1997). On the test day, weight matched pairs of rats (\pm 5%), unfamiliar to each other, were given identical treatments and returned to their home cages. Animals were randomly divided into 5 treatment groups, with 5 pairs per group, and were given one of the following i.p. treatments: Compound 10 (3, 10 or 30 mg/kg), vehicle (1 ml/kg) or chlordiazepoxide (5 mg/kg). Dosing was 1 hour prior to testing. Rats were subsequently placed in a white perspex test box or arena (54 x 37 x 26 cm), whose floor was divided up into 24 equal squares, for 15 minutes. An air conditioner was used to generate background noise and to keep the room at approximately 74°F. All sessions were videotaped using a JVC camcorder (model GR-SZ1, Elmwood Park, NJ) with either TDK (HG ultimate brand) or Sony 30 minute videocassettes. All sessions were conducted between 1:00 - 4:30 P.M. Active social interaction, defined as grooming, sniffing, biting, boxing, wrestling, following and crawling over or under, was scored using a stopwatch (Sportsline model no. 226, 1/100 sec. discriminability). The number of episodes of rearing (animal completely raises up its body on its hind limbs), grooming (licking, biting, scratching of body), and face washing (i.e. hands are moved repeatedly over face), and number of squares crossed were scored. Passive social interaction (animals are lying beside or on top of each other) was not scored. All behaviors were assessed later by an observer who was blind as to the treatment of each pair. At the end of each test, the box was thoroughly wiped with moistened paper towels.

30

Animals

Male albino Sprague-Dawley rats (Taconic Farms, NY) were housed in pairs under a 12 hr light dark cycle (lights on at 0700 hrs.) with free access to food and water.

35

Drug Administration

Compound 10 was dissolved in 5% lactic acid. Chlordiazepoxide (purchased from Sigma Chemical Co., St. Louis, MO) was dissolved in distilled water. The vehicle 5 was an equal mixture of 5% lactic acid and distilled water. All drug solutions were made up 10 minutes prior to injection and the solutions were discarded.

Data Analysis

10 The social interaction data (time interacting, rearing and squares crossed) were subjected to a randomized, one-way ANOVA and post hoc tests conducted using the Student-Newman-Keuls test. The data were subjected to a test of normality (Shapiro-Wilk test). The data were analyzed 15 using the GBSTAT program, version 6.5 (Dynamics Microsystems, Inc., Silver Spring, MD, 1997). All data are presented as means \pm S.E.M.

20

Results and Discussion

Cloning and Sequencing

Discovery of an Expressed Sequence Tag (EST) F07228 in
5 GENEMBL Homologous to FB41a

A BLAST search of GENEMBL with a Synaptic Pharmaceutical Corporation proprietary sequence, FB41a, resulted in the identification of an EST (accession number F07228) with a high degree of homology to FB41a and somatostatin, opiate and galanin receptors.

10 Construction and Screening of a Human Hippocampal cDNA
15 Library

A human hippocampal cDNA library containing a total of 2.2 x10⁶ independent clones with a mean insert size of 3.0 kb was prepared in the expression vector pEXJ.BS. The library was plated on agar plates (ampicillin selection) and glycerol stocks for 450 pools of 5000 independent clones were prepared. Primary glycerol stocks were also grouped together in groups of approximately 10 to create superpools.

20 Cloning of the full-length sequence of MCH1

Glycerol stocks of the superpools and primary pools from the human hippocampal cDNA library were screened by PCR with F07228 specific primers T579 and T580. One positive primary pool 490, was successively divided into subpools, amplified in LB medium overnight and screened by PCR using primers T579 and T580. One positive subpool, 490-4-10-23 was plated on agar plates (ampicillin selection), and colonies were transferred to nitrocellulose membranes (Schleicher and Schuell, Keene, NH). Filters were hybridized for two days under high stringency conditions with 10⁶ cpm/ml of a ³²P-labeled cDNA probe, T581, designed against the F07228 EST sequence. Filters were

washed and apposed to Biomax MS film (Kodak). Seven positive colonies were picked, streaked on LB-AMP plates, and grown overnight. Two individual colonies from each of the original seven were picked and subjected to vector-anchored PCR using the following primer pairs: T95, T580 and T94, T579. One positive colony, G1, was amplified overnight in TB and processed for plasmid purification. This plasmid was designated TL230 and sequenced on both strands. Nucleotide and peptide sequence analysis were performed with GCG programs (Genetics Computer Group, Madison, WI). A *Hind*III- *Kpn*I fragment of TL230 was subcloned into the mammalian expression vector pEXJ, and named TL231. The largest open reading frame in this construct contains 1266 nucleotides (Figure 1), which is predicted to encode a protein of 422 amino acids (Figure 2). There are three in-frame methionines in the amino terminus which could result in a protein of 422, 417 or 353 amino acids. Hydropathy analysis of the protein is consistent with a putative topography of seven transmembrane domains, indicative of the G protein-coupled receptor family (Figure 3). TL231 has been named MCH1.

Database analysis of the sequence of MCH1 revealed that it was most similar to somatostatin receptors. Further database analysis revealed a Genbank submission (accession number AF008650, deposited on October 1, 1997) which appears to be the rat homologue of TL231. AF008650 is 69 nucleotides shorter than MCH1 at the 5'end, and predicts a different initiating methionine. Figures 4 and 5 illustrate the nucleotide and amino acid sequence for the rat MCH1 receptor, respectively.

Inositol phosphate response of MCH1-transfected cells

The expression vector (pEXJ) containing the MCH1 cDNA was transfected by electroporation into Cos-7 cells in

combination with an expression vector (pEXJ) containing the G_{α16} subunit. After plating and labeling with [³H]-myo-inositol, the transfectants were challenged with a ligand library that included, among other things, melanin 5 concentrating hormone (MCH) (10 μM final concentration) and then assayed for inositol phosphate (IP) formation. In five out of the seven screens, cells transfected with MCH1 (with G_{α16}) gave an approximately 1.4-fold increase in IP production as compared to cells transfected with G_{α16} alone 10 when challenged with MCH.

Subsequent experiments demonstrated that 10 μM MCH was able to stimulate IP release 3.4-fold over basal levels in Cos-7 cells transfected with MCH1 alone, suggesting that 15 this receptor couples through the G_q signaling pathway. The IP response was shown to be dose-dependent to MCH with an EC₅₀ value of 9.3 ± 1.7 nM (n=2) and an E_{max} of approximately 400% basal (404 ± 72) (Figure 6).

Several additional compounds were tested for their ability 20 to activate MCH1. No dose-responsiveness of inositol phosphate formation could be detected in Cos-7 cells transfected with MCH1 when challenged with somatostatin, haloperidol, or dynorphin A1-13, discounting the possibility that MCH1 encodes a somatostatin-like or 25 opioid-like or sigma-like GPCR subtype (Figure 7)

Microphysiometric response of MCH1-transfected cells to MCH

CHO cells were transiently transfected with MCH1 using 30 lipofectant, challenged with increasing concentrations of MCH or Phe¹³,Tyr¹⁹-MCH, and subsequently monitored for changes in extracellular acidification rates. Both ligands produced a dose-dependent increase in acidification rate with an EC₅₀ value of 8.6 nM for MCH and 35

51.8 nM for Phe¹³,Tyr¹⁹-MCH. Neither native CHO cells or mock (pEXJ) transfected CHO cells exhibited a change in acidification rate when exposed to MCH or Phe¹³,Tyr¹⁹-MCH (Figure 8).

5

Transcriptional response of MCH1-transfected cells

Cos-7 cells were transiently transfected with MCH1 and a c-fos-β-gal reporter construct by the DEAE-dextran method. The cells were challenged with assorted drugs, including 10 MCH, and transcriptional activity measured by colorimetric assay of β-galactosidase protein expression. Initial single dose challenges with MCH at a concentration of 10 μM stimulated c-fos-regulated transcriptional activity approximately 3.9-fold over cells challenged with medium only. Cells transfected with only the c-fos-β-gal construct showed no response to MCH. Subsequent experimentation showed the transcription activation response to be dose-dependent to MCH with an EC₅₀ value of 116 nM (Figure 9).

20

Binding of [¹²⁵I]Phe¹³,Tyr¹⁹-MCH in MCH1-transfected cells

Membranes harvested from Cos-7 cells transfected with MCH1 by the DEAE-dextran method exhibited specific binding for [¹²⁵I]Phe¹³-Tyr¹⁹-MCH (about 80 fmol/mg membrane protein) over mock-transfected cells (about 20 fmol/mg membrane protein) at 0.1 nM radioligand concentration. Specific [¹²⁵I]Phe¹³-Tyr¹⁹-MCH binding was about 70% of total binding at a radioligand concentration of 0.1 nM (Figure 10).

25

Localization of mRNA encoding human MCH1 receptors

RT-PCR was used to assess the presence of MCH1 receptor encoding message in mRNA samples isolated from a variety of human tissues (Table 1, Figure 11). After amplification, PCR reactions were size fractionated on 10%

polyacrylamide gels, and stained with SYBR Green I. Images were analyzed using a Molecular Dynamics Storm 860 workstation. The amplified band corresponding to MCH1 receptor (490 base pairs) is indicated (arrow). RT-PCR analysis indicates the distribution of mRNA encoding human MCH1 receptor is widespread throughout all tissues assayed, including both central nervous system tissue and peripheral organs. This widespread distribution implies broad regulatory functions that involve nervous system as well as endocrine mechanisms.

Table 1. Distribution of mRNA coding for human MCH1 receptors.

Region	human MCH 1	Potential applications
liver	+++	Diabetes
kidney	+++	Hypertension, Electrolyte balance
lung	+++	Respiratory disorders, asthma
heart	+++	Cardiovascular indications
small intestine	+++	Gastrointestinal disorders
striated muscle	+++	Musculoskeletal disorders
pituitary	+++	Endocrine/neuroendocrine regulation
whole brain	+++	
amygdala	+++	Depression, phobias, anxiety, mood disorders
cerebral cortex	+++	Sensory and motor integration, cognition
hippocampus	+++	Cognition/memory
hypothalamus	+++	appetite/obesity, neuroendocrine regulation
spinal cord	+++	Analgesia, sensory modulation and transmission
cerebellum	+++	Motor coordination
thalamus	+++	sensory integration
substantia nigra	+++	Modulation of dopaminergic function. Modulation of motor coordination.
caudate-putamen	+++	Modulation of dopaminergic function
fetal brain	+++	Developmental disorders
fetal lung	+++	Developmental disorders

fetal kidney	+++	Developmental disorders
fetal liver	+++	Developmental disorders

The cloning of the gene encoding the human MCH1 receptor
5 has provided the means to explore its physiological role
by pharmacological characterization, and by Northern and
in situ mapping of its mRNA distribution. Further, the
availability of the DNA encoding the human MCH1 receptor
will facilitate the development of antibodies and
10 antisense technologies useful in defining the functions of
the gene products *in vivo*. Antisense oligonucleotides
which target mRNA molecules to selectively block
translation of the gene products *in vivo* have been used
successfully to relate the expression of a single gene
15 with its functional sequelae. Thus, the cloning of this
receptor gene provides the means to explore its
physiological role in the nervous system and elsewhere,
and may thereby help to elucidate structure/function
relationships within the GPCR superfamily.

20

The presence of three different potential starting codons
in the cDNA sequence of TL231 opens the question of which
of the possible transcripts yields an active MCH receptor.
In order to establish whether a transcript of the first
25 and second starting codons of TL231 encode a functional
human MCH receptor, methionines 6 and 70 of TL231 were
mutated to alanine (construct R114; See Figure 12). The
third methionine at position 70 was also mutated to an
alanine (construct R106; See Figure 12). Transfections of
30 TL231, R106 or R114 into COS-7 cells all resulted in MCH-
mediated increases of intracellular calcium, as measured
by a fluorescent intensity plate reader in cells loaded
with the calcium dye fluo-3 (FLIPR, Molecular Devices).
As shown in Table 2, COS-7 cells transfected with TL231,

R106, R114 and BO120 showed dose-related mobilization of intracellular calcium when exposed to increasing concentrations of MCH with similar maximal responses and EC₅₀ values. These data demonstrate that transcripts starting at the first and/or second and third methionine of TL231 encode a functional human MCH receptor.

Table 2.

Transfected Construct	Response to Melanin Concentrating Hormone*	
	EC ₅₀ (nM)	Max. Response (RFU**)
TL231	60, 12	3,535, 14,000
R114	98, 9	2,267, 1,550
R106	85, 55	4642, 2000
BO120	12, 3.5	30,000, 25,000

*Results from two independent experiments

** RFU = relative fluorescence units

Discovery of MCH1 Receptor Antagonists

The intracellular calcium response to MCH in COS-7 cells transfected with MCH1 was used as an assay to identify MCH1 receptor antagonists. Compounds of known chemical structure were added at a concentration of 1 mM to COS-7 cells expressing MCH1 loaded with the calcium indicator fluo-3, and the fluorescence intensity was measured in the absence and presence of 500 nM MCH. MCH1 antagonist compounds were identified by their ability to inhibit the MCH-elicited response. The identified compounds were then

tested at 12 different concentrations (between 1e-4 to 3e-10 M) to determine the dose that inhibited the response of 500 nM MCH by 50% (IC₅₀). From the IC₅₀ values, the antagonist potency (K_b) was derived using the Cheng-Prussoff correction (Lazareno and Birdsall, 1993). Table 3 exemplifies compounds that were found to have a K_b lower than 500 nM.

Among the compounds tested, Compound 10 was identified as the most potent antagonist of the human MCH1 receptor. The antagonism of Compound 10 was further characterized with inositol phosphate response in Cos-7 cells transfected with the human MCH1 receptor. As shown in Figure 16, in the presence of 1, 3, and 10 nM of Compound 10 parallel displacement of the dose-response curves for MCH were observed, suggesting the presence of a competitive antagonist. The Schild analysis of the dose-response yielded a pA₂ = 9.24 with a slope close to unity. This value correlates closely with the K_b = 0.3 nM determined using the intracellular calcium mobilization assay.

Given the high affinity of Compound 10 for the MCH1 receptor, a tritiated analog of this compound was synthesized. [³H]Compound 10 was tested for its ability to bind to membrane preparations of cells expressing the human MCH1 receptor. As shown in Figure 17, addition of increasing concentrations of [³H]Compound 10 in the absence (Total) and presence of 10 mM Compound 10 (Nonspecific) resulted in saturable specific binding to membrane preparations of Cos-7 cells transfected with MCH1. The Scatchard analysis of the binding data estimated a K_d = 0.18 nM for [³H]Compound 10 and maximum number of

binding sites (B_{max}) = 870 fmol/mg protein (see inset of Figure 17). In competition binding assays using membrane preparations of Cos-7 cells transfected with MCH1, Compound 10 and MCH completely displaced the specific
5 binding of [3 H]Compound 10 with IC₅₀'s of 0.33 and 511 nM respectively (Figure 18). In non-transfected Cos-7 cells the binding of [3 H]Compound 10 was not displaced by MCH or unlabeled Compound 10 up to 10 mM. These data together demonstrate that [3 H]Compound 10 is a specific and high
10 affinity radioligand for the MCH1 receptor.

As described in the Background of the Invention, compounds that block the effects of MCH on its receptor can potentially be used for the treatment of eating disorders and obesity. The study of the regulation of body weight and food intake point towards an important role for hypothalamic circuits and their neurotransmitters together with circulating metabolic signals, such as leptin, in energy homeostasis (Elmquist et al., 1999). Hypothalamic neurons that mediate appetite-driving (orexigenic) effects include those that use neuropeptide Y, MCH, galanin, and orexin as transmitters. Conversely, neural elements that mediate orexigenic signals include among others those that use serotonin (5HT), alpha-MSH, and CART (cocaine and amphetamine regulated transcript) as transmitters. Recent
15 advances in the molecular cloning of the receptors for some of these transmitter molecules, together with the characterization of their pharmacological properties, has enabled the identification of numerous potential targets for therapeutic intervention. In the case of NPY, the evidence suggests that both NYP1 and NPY5 receptors are involved in mediating the orexigenic effects of NPY (Inui,
20 1999). The antiorexigenic effects of alpha-MSH are mediated by the MC4 receptor (Fan et al, 1997). In addition, an important role for the antiorexigenic effects
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of serotoninergic agonists was suggested by the obese phenotype of mice with targeted deletion of the 5HT2C receptor gene (Nonogaki, 1998). However, the interaction between orexigenic and anorexigenic pathways in the hypothalamus displays considerable redundancy typical of other biological control mechanisms (Kalra et al., 1999). This complexity suggests that the design of development of drugs that target multiple orexigenic receptors might result in an effective therapeutic modality that could restore the imbalance of energy homeostasis that results in obesity. One such approach could involve the administration of a combination of antagonists of the MCH1 receptor such as those described above together with NPY1, NPY5, or galanin receptor antagonists. An alternative approach is to design a single molecule that antagonizes the orexigenic effects of one or more of MCH, NPY, and galanin by binding to one or more of MCH1, NPY1, NPY5, or galanin receptors. However, in either case the compound(s) should be free of antagonist activity at the MC-4 or 5HT2C receptor, since antagonizing these receptors could result in increased food intake and obesity (Fan et al., 1997). The design of such compounds can be optimized by determining their binding affinity at the recombinant MCH1, NPY1, NPY2, Gal1, Gal2, Gal3, 5HT2C, and MC-4 receptors. The methods to obtain the cDNA of the receptor, express said receptors in heterologous systems, and carry out assays to determine binding affinity are described in the following publications: human NPY1 (Larhammar et al., 1992), human NPY5 (U.S. Patent No. 5,602,024, the disclosure of which is hereby incorporated by reference in its entirety into this application), human Gal1 (Habert-Ortoli et al., 1994), human Gal2 (Smith et al., 1997), human Gal3 (Smith et al., 1998), rat 5HT2C (Julius et al., 1988), and human MC-4 (Gantz et al., 1993). Additionally, the compounds would optimally not

bind at the following receptors due to possible side effects: human H1 histamine, human H2 histamine, human alpha-1A adrenergic, human alpha-1D adrenergic, human alpha-2A, human alpha-2B adrenergic, human alpha-2C adrenergic, human dopamine D1, D2, D3, D5 receptors and the β -adrenoceptor. Binding studies for the β -adrenoceptor may be performed according to the method of Riva and Creese, 1989. Binding assays for the remainder of the receptors may be carried out according to the procedures described in U.S. Patent No. 5,780,485, the disclosure of which is hereby incorporated by reference in its entirety into this application.

As further described in the Background of the Invention, compounds that block the effects of MCH on the MCH1 receptor can potentially be used for the treatment of depression and anxiety. Biogenic amine transmitter molecules that mediate neuronal signals are currently known in the art and include among others serotonin (5HT), norepinephrine (NE), and dopamine (DA). Recent advances in the molecular studies of the mechanisms for these transmitter molecules, together with the characterization of their pharmacological properties, has enabled the identification of numerous potential targets for therapeutic intervention. Inhibitors of the 5HT, NE and DA transporter systems, and inhibitors of the enzyme, monoamine oxidase, have been widely studied and are known to enhance the action of biogenic amine neurotransmitters. The resultant clinically effective antidepressant drugs are known today as TCAs, SSRIs and MAOIs. (Tatsumi et al., 1997; Iversen, 2000).

In the case of MCH, the evidence presented in this invention suggests that GPCR-targeted molecules that bind

to and antagonize the MCH1 receptor may be used for the treatment of depression and/or anxiety disorders. However, the MCH1 antagonist(s) should be free of activity at 5HT, NE and DA transporters. Furthermore, the MCH1 antagonist(s) should not inhibit the enzymatic activity of monoamine oxidase A (MAO_A) or monoamine oxidase B (MAO_B) present in the brain (i.e. central MAO). The design of such compounds can be optimized by determining their binding affinity at the 5HT, NE and DA transporters in tissue assays. The design of such compounds can be further optimized by determining their interaction with central MAO_A and central MAO_B .

Additionally, the MCH1 antagonist(s) would optimally not bind at the following receptors due to possible side effects: human H_1 histamine; human H_2 histamine; human α_{1A} adrenergic, human α_{1B} adrenergic, human α_{1D} adrenergic, human α_{2A} adrenergic, human α_{2B} adrenergic, and human α_{2C} adrenergic; human dopamine D₁, D₂, D₃, D₄, and D₅; and the human 5HT_{1A}, human 5HT_{1B}, human 5HT_{1D}, human 5HT_{1E}, human 5HT_{1F}, human 5HT_{2A}, rat 5HT_{2C}, human 5HT₄, human 5HT₆, and human 5HT₇ receptors.

Radioligand Binding Assays and Enzymatic Assays

The methods to obtain the cDNA of the receptors, express said receptors in heterologous systems, and carry out assays to determine binding affinity are described as follows.

Human 5HT_{1B}, 5HT_{1D}, 5HT_{1E}, 5HT_{1F}, and 5HT₇ Receptors: The cell lysates of LM(tk) clonal cell line stably transfected with the genes encoding each of these 5HT receptor subtypes were

prepared as described above. Cell membranes were suspended in 50mM TrisHCl buffer (pH 7.4 at 37°C) containing 10 mM MgCl₂, 0.2 mM EDTA, 10 M pargyline, and 0.1% ascorbate. The affinities of compounds were determined in equilibrium competition binding assays by incubation for 30 minutes at 37 °C in the presence of 5 nM [³H]serotonin. Nonspecific binding was determined in the presence of 10 μM serotonin. The bound radioligand was separated by filtration through GF/B filters using a cell harvester.

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Human 5HT_{2A} Receptor: The coding sequence of the human 5HT_{2A} receptor was obtained from a human brain cortex cDNA library, and cloned into the cloning site of pCEXV3 eukaryotic expression vector. This construct was transfected into COS7 cells by the DEAE dextran method (Cullen, 1987). Cells were harvested after 72 hours and lysed by sonication in 5 mM TrisHCl, 5 mM EDTA, pH 7.5. The cell lysates were subjected to centrifugation at 1000 rpm for 5 minutes at 4°C, and the supernatant was subjected to centrifugation at 30,000 × g for 20 minutes at 4°C. The pellet was suspended in 50 mM TrisHCl buffer (pH 7.7 at room temperature) containing 10 mM MgSO₄, 0.5 mM EDTA, and 0.1% ascorbate. The affinity of compounds at 5HT_{2A} receptors were determined in equilibrium competition binding assays using [³H]ketanserin (1 nM). Nonspecific binding was defined by the addition of 10 μM mianserin. The bound radioligand was separated by filtration through GF/B filters using a cell harvester.

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5HT_{1A} Receptor: The cDNA corresponding to the 5HT_{1A} receptor open reading frames and variable noncoding 5' and 3' regions, was cloned into the eukaryotic expression

vector pCEXV3. These constructs were transfected transiently into COS7 cells by the DEAEdextran method (Cullen, 1987), and harvested after 72 hours. Radioligand binding assays were performed as described above for the 5HT_{2A} receptor, except that ³H8OHDPAT was used as the radioligand and nonspecific binding was determined by the addition of 10 μM mianserin.

Other 5HT Receptors: Other serotonin receptor binding assays were performed according to published methods: rat 5HT_{2C} receptor (Julius et al., 1988); and 5HT₆ (Monsma, et al., 1993). The binding assays using the 5HT₄ receptor were performed according to the procedures described in U.S. Patent No. 5,766,879, the disclosure of which is hereby incorporated by reference in its entirety into this application.

Other receptors: Cell membranes expressing human dopamine D₁, D₂, D₄ and rat D₃ receptors were purchased through BioSignal, Inc. (Montreal, Canada). Binding assays using the histamine H₁ receptor; dopamine receptors; and α_{1A}, α_{1B}, and α₂ adrenergic receptors may be carried out according to the procedures described in U.S. Patent No. 5,780,485, the disclosure of which is hereby incorporated by reference in its entirety into this application. Binding assays using the dopamine D₅ receptor may be carried out according to the procedures described in U.S. Patent No. 5,882,855, the disclosure of which is hereby incorporated by reference in its entirety into this application. Binding assays for the human α_{1D} adrenergic receptor may be carried out according to the procedures described in U.S. Patent No. 6,156,518, the disclosure of which is hereby incorporated by reference in its entirety into this application.

The methods to determine binding affinity at native
transporters are described in the following publications:
5HT transporter and NE transporter (Owens et al., 1997),
5 and DA transporter (Javitch et al, 1984).

The methods to determine activity at monoamine oxidase
enzymes (for example, central MAO_A and MAO_B) are described
by Otsuka and Kobayashi, 1964.

Table 3a: Antagonist potency (K_b) at the human MCH1 receptor, and binding affinity (K_i) at NPY, galanin and 5HT2C receptors.

Compound	hMCH1	hNPY1	hNPY5	hGALR1	hGALR2	hGALR3	r5HT2C
	K_b (nM)	K_i (nM)					
10	0.3	>50000	>50000	>50000	>50000	>50000	29,585
18	1	>50000	>50000	>50000	>50000	>50000	32,617
14	2	ND	ND	>50000	42,603	>50000	663
20	5	27,076	>50000	>50000	>50000	>50000	15,058
19	7	>50000	>50000	>50000	>50000	>50000	11,720
29	9	>50000	46,075	>50000	>50000	>50000	>50000
2	18	ND	ND	>50000	>50000	>50000	39,837
6	42	6,667	4,735	11,057	14,921	21,095	25,549
1	42	>50000	>50000	>50000	>50000	>50000	>50000
28	60	>50000	>50000	>50000	>50000	>50000	34,087
25	126	>50000	>50000	>50000	>50000	>50000	41,009
37	162	>50000	>50000	>50000	>50000	>50000	>50000
4	187	>50000	>50000	>50000	>50000	>50000	34,798
26	260	>50000	>50000	>50000	>50000	>50000	2,900
27	279	>50000	>50000	>50000	>50000	>50000	>50000
13	284	9,601	>50000	11,262	4,727	5,985	25,030
30	479	>50000	>50000	>50000	>50000	>50000	8,859

Table 3b: Antagonist potency (Kb) at the human MCH1 receptor, and binding affinity (Ki) at human MCH1, NPY1, NPY5, GALR1, GALR2, GALR3, and rat 5HT2C receptors.

Compound	hMCH1	hMCH1 *	hNPY1	hNPY5	hGALR1	hGALR2	hGALR3	r5HT2C
	Kb (nM)	Ki (nM)						
10	0.3	0.08	>50000	>50000	>50000	>50000	>50000	29,585
19	7	3	>50000	>50000	>50000	>50000	>50000	11,720
18	1	4	>50000	>50000	>50000	>50000	>50000	32,617
20	5	6	27,076	>50000	>50000	>50000	>50000	15,058
1	42	40	>50000	>50000	>50000	>50000	>50000	>50000
2	18	49	ND	ND	>50000	>50000	>50000	39,837
14	2	50	ND	ND	>50000	42,603	>50000	663
4	187	131	>50000	>50000	>50000	>50000	>50000	34,798
13	284	171	9,601	>50000	11,262	4,727	5,985	25,030
29	9	350	>50000	46,075	>50000	>50000	>50000	>50000
6	42	463	6,667	4,735	11,057	14,921	21,095	25,549

* Binding affinity (Ki) was determined in competition binding assays using membrane preparations of A293 cells expressing the human MCH1 receptor and [³H]Compound 10 as the radioligand.

Table 3c: Binding affinities (Ki) at the rat MCH1, human Dopamine D2, human Histamine H1 and human Alpha-1a Adrenergic receptors.

Compound	rMCH1 Ki (nM)	hD2 Ki (nM)	hH1 Ki (nM)	hAlpha-1a Ki (nM)
38	1.34	2370.49	378.91	23.82
39	3.33	25,142.39	5664.28	25.48
40	2.72	2651.67	7123.16	8.72
41	0.04	9605.79	4541.69	14.31
42	0.6	3274.88	7795.17	10.91
43	0.23	3570.6	10,774.03	21.86
44	0.09	3607.95	2594.67	11.39
45	14.69	>50000	4432.68	6027.11
46	8.16	>50000	2867.82	2424.16
47	34.28	13,540.64	3251.17	553.45
48	22.15	>50000	7769.8	16,563.03
49	225.47	ND	ND	ND
50	13.74	19,796.44	7468.06	10,385.44
51	0.79	6638.62	1230.84	10.05
52	0.81	165.91	1428.58	200.24
53	50.76	3447.38	10,387.86	2307.97
54	29.87	22,966.69	11,408.54	11,120.65
55	203.74	ND	ND	ND
56	0.26	10,399.66	7228.37	305.44
57	90	6092	823	49
58	3.9	2839	700	32.1

Table 3c: Binding affinities (K_i) at the rat MCH1, human Dopamine D2, human Histamine H1 and human Alpha-1a Adrenergic receptors.

	59	768	ND	ND	ND
60	357	ND	ND	ND	ND
61	14.2	1139	1618	9.1	ND
62	274	ND	ND	ND	ND
63	1000	ND	ND	ND	ND
64	627	ND	ND	ND	ND
65	69	1430	1733	26.4	ND
66	2.8	862	461	19.4	ND
67	197	ND	ND	ND	ND
68	84	771	571	57	ND
69	11.9	551	ND	ND	ND
70	167	ND	ND	ND	ND
71	720	ND	ND	ND	ND
72	272	ND	ND	ND	ND
73	342	ND	ND	ND	ND
74	29.5	782	ND	115	ND
75	506	ND	ND	ND	ND
76	21	470	ND	41.3	ND
77	630	ND	ND	ND	ND
78	52	5181	2277	284	ND
79	1036	ND	ND	ND	ND
80	67	1252	ND	127	ND
81	463	ND	ND	ND	ND

Table 3c: Binding affinities (K_i) at the rat MCH1, human Dopamine D2, human Histamine H1 and human Alpha-1a Adrenergic receptors.

	82	192	1977	ND	516
83	91	503	ND	ND	130
84	511	ND	ND	ND	ND
85	654	ND	ND	ND	ND
86	382	ND	ND	ND	ND
87	362	ND	ND	ND	ND
88	160	ND	ND	ND	ND
89	615	ND	ND	ND	ND
90	651	ND	ND	ND	ND
91	11.5	9654	2000	533	—
92	62	12,026	2454	1489	—
93	29.1	34,993	16,734	1087	—
94	18.2	>50000	6595	1592	—
95	11.8	>50000	6401	2937	—
96	50	7451	273	12.3	—
97	946	ND	ND	ND	—
98	118	ND	ND	ND	—
99	12	10,428	2560	434	—
100	11.5	8673	11,092	704	—
101	1.6	42.2	3.4	18	—
102	187	ND	ND	ND	—
103	52	>50000	36,907	>50000	—
104	6.7	735	6390	452	—

Table 3c: Binding affinities (K_i) at the rat MCH1, human Dopamine D2, human Histamine H1 and human Alpha-1a Adrenergic receptors.

	105	7.1	471	39.1	140
106	3.9	1077	304	161	
107	3.1	152	130	33.5	
108	3.8	244	264	13.2	
109	7.1	191	1320	221	
110	4.9	83	283	187	
111	5	162	1100	125	
112	22.3	435	32.5	55	
113	16.6	41,994	48,658	3206	
114	20.1	390	590	233	
115	12.9	262	46.9	49.1	
116	0.923	52	546	22.3	
117	13.6	281	969	310	
118	12.8	319	25,320	719	
119	22.4	766	25,307	1058	
120	14.8	313	6994	1142	
121	17	331	9390	1720	
122	3.3	132	3473	944	
123	5.9	133	2146	511	
124	9.3	66	329	204	
125	32.5	46.6	>50000	232	
126	50	1050	7998	1521	
127	6.6	119	1710	226	

	128	31.4	41,454	33,096	645
129	22.3	41,454	6522	381	
130	48.6	39,511	1862	333	
131	11.8	19,041	2844	2469	
132	44.6	41,454	39,710	10,965	
133	25.7	447	4178	167	
134	22.2	37.6	>50000	1313	
135	19.4	244	507	722	
136	14.3	833	9789	620	
137	377	ND	ND	ND	
138	11.2	ND	ND	ND	
139	48.1	ND	ND	ND	
140	121	ND	ND	ND	
141	3.2	2449	3816	3021	

Table 3d: Antagonist binding affinity (K_i) at the human MCH1 receptor vs. alpha-adrenergic and dopamine receptors.

Compound	hMCH1	h α_{1A}	h α_{1B}	h α_{1D}	h α_{2A}	h α_{2C}	hD ₁	hD ₂	xD ₃	hD ₄	hD ₅
	K_i (nM)	K_i (nM)	K_i (nM)	K_i (nM)	K_i (nM)	K_i (nM)	K_i (nM)	K_i (nM)	K_i (nM)	K_i (nM)	K_i (nM)
10	0.08	45.8	11343	12334	ND	ND	1928	*	5890	*	773
19	3	2.5	104	517	285	349	174	ND	ND	ND	ND
18	4	1.7	418	612	57	74	96	596	624	1216	ND
20	6	0.3	407	207	99	97	138	ND	ND	ND	ND
1	40	2214	9033	1307	ND						

Table 3e: Antagonist binding affinity (K_i) at the human MCH1 receptor vs. serotonin and histamine receptors.

Compound	hMCH1	h5HT _{1A}	h5HT _{2A}	hH ₁	hH ₂
	K_i (nM)	K_i (nM)	K_i (nM)	K_i (nM)	K_i (nM)
10	0.08	ND	ND	4875	*
19	3	7943	ND	11	ND
18	4	1245	1567	31	944
20	6	2188	2818	39	1905

* = >50000

ND = Not determined

AUTORADIOGRAPHIC DISTRIBUTION OF MCH1 RECEPTOR BINDING SITES IN THE RAT CNS

Telencephalon

5 A low density of MCH1 receptor binding sites was detected in the cerebral cortex with slightly increased binding in the superficial layers. The septal nuclei (Figure 20A, C), claustrum (Cl) (Figure 20A, B), ventral and horizontal limbs of the diagonal band, and piriform cortex (Pir) 10 likewise contained a low density of MCH1 receptor binding sites (Figures 20A, A-F; 20B, G and H).

Some of the highest MCH1 receptor binding in the rat CNS was observed in the basal ganglia and the olfactory 15 tubercle (Tu) (Figure 20A, B). The caudate-putamen (CPu) and core of the accumbens nucleus (AcbC) displayed dense labeling of MCH1 receptors while a very intense labeling was present in the shell of the accumbens nucleus (Acbsh) 20 (Figure 20A, B). The globus pallidus (GP) was unlabeled. The subthalamic nuclei (STh), part of the basal ganglia circuit, was moderately labeled (Figures 20A, F).

The amygdala and extended amygdala displayed a moderately low labeling with slightly higher radioligand binding 25 observed in the bed nucleus of the stria terminalis (BSTm) (Figure 20A, C), the basolateral (BLA) and lateral amygdaloid nuclei (LA) (Figures 20A, D and F).

Diencephalon

In general, MCH1 receptor binding was weak throughout the diencephalon. In the thalamus there was a slight increase in binding intensity in the paraventricular (PVA), centromedial, and anterodorsal thalamic nuclei (AD) (Figure 5 20A, D). In the epithalamus the medial habenular nucleus (MHb) contained MCH1 receptor binding sites (Figure 20B, G). Throughout the hypothalamus there was a uniformly weak binding signal (Figures 20A, C-H; 20B, G and H). There was a slight increase in MCH1 binding intensity in the 10 ventromedial hypothalamus (VMH) and in the medial mammillary nucleus (MM) (Figures 20A, E; 20B H).

MCH1 receptor binding was moderate in the induseum griseum (IG) (Figure 20A, B) and in Ammon's horn of the hippocampal formation (CA1, CA2, CA3) (Figures 20A, E and F). MCH1 15 binding sites were present in the stratum oriens (so) and stratum radiatum (sr) of field CA1, and in the stratum oriens of field CA3. Moderate binding was observed in the molecular layer of the dentate gyrus and in the 20 pre/parasubiculum (Figures 20A, E and H).

Mesencephalon

Overall, MCH1 receptor binding in the mesencephalon was very weak. A slight increase in binding intensity was 25 evident in the periaqueductal gray (PAG) and in the pontine nuclei (Pn) (Figures 20B, I and J). Moderate binding was observed in the superior colliculus (SC) and the dorsal raphe nucleus (DR) (Figures 20B, I and J).

The highest density of MCH1 receptor binding sites in the rhombencephalon was seen in the locus coeruleus (LC) (Figure 20B, L). There was consistently low MCH1 receptor binding throughout the pons and medulla (Figures 20B, K and L). Slightly higher binding was detected in the inferior colliculus (IC), the dorsal tegmental nuclei (DTN) and parabrachial nuclei (PB) (Figure 20B, K) and the lateral superior olive (LSO) (Figure 20B, L).

10 Spinal cord

MCH1 receptor binding sites appeared to be uniformly distributed throughout the dorsal and ventral horns of the spinal cord (Figure 20B, M). Binding density was slightly increased in the superficial dorsal horn.

15

Table 4

The distribution of MCH1 receptor binding sites in the rat CNS using Receptor Autoradiography with 0.1 nM [³H]Compound 10 in the presence of 1 μM prazosin and 100 μM dopamine. 20 The strength of [³H]Compound 10 (MCH1) labeling intensity for the various rat brain regions was graded as absent (-), weak (+), moderate (++) , heavy (+++), or intense (++++) .

	Region	Density of MCH1 receptor binding sites	Potential Application
5	Olfactory System		Modulation of olfactory sensation
	Anterior olfactory n.	+	
	Olfactory tubercle	+++	
	Islands of Calleja, major	+++	
	Telencephalon		Cognition
	Clastrum	++	Visual attention
	Dorsal endopiriform n.	+	Olfactory information processing
	Basal Ganglia		
	Globus pallidus	-	
	Caudate-putamen	+++	Sensory/motor integration
10	Accumbens n., shell	++++	Treatment of drug addiction. This region is particularly sensitive to psychoactive drugs.
	Accumbens n., core	+++	Treatment of schizophrenia, anxiety and/or depression.

Region	Density of MCH1 receptor binding sites	Potential Application
Medial septal n.	+	Cognitive enhancement via cholinergic system
Septohippocampal n.	+	
Amygdala		Modulation of endocrine functions and integrated behaviors such as defense, ingestion, reproduction, and learning. Treatment of anxiety and/or depression.
Central amygdaloid n.	+	Fear and anxiety
Basolateral amygdaloid n.	+	Olfaction
Bed n. of the stria terminalis	++	Modulation of the limbic system. Treatment of anxiety and/or depression.
Anterior cortical n.	+	Olfaction
Diencephalon		

Region	Density of MCH1 receptor binding sites	Potential Application
Thalamus		Analgesia/ Modulation of sensory information
Paraventricular n.	+	Modulation of motor and behavioral responses to pain
Centromedial n.	+	Modulation of motor and behavioral responses to pain
Anterodorsal n.	+	Modulation of motor information to the cerebral cortex/ eye movement
Reticular n.	+	Alertness/sedation
Mediodorsal n.	-	

Region	Density of MCH1 receptor binding sites	Potential Application
Hypothalamus	+	Regulation of endocrine function, reproductive behaviors, and appetite/obesity. Treatment of anxiety and/or depression.
Hippocampal formation		Cognition/memory consolidation and retention
CA1	++	
CA2	++	
CA3	++	
Pre/parasubiculum	++	Modulation of memory acquisition
Mesencephalon		
Superior colliculus	++	Modulation of visual information/spatial localization
Pontine n.	+	
Periaqueductal gray	+	Analgesia
Substantia nigra	+	
Interpeduncular n.	++	Analgesia
Caudal linear raphe n.	+	

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Region	Density of MCH1 receptor binding sites	Potential Application
Locus coeruleus	++	Modulation of NA transmission
Cerebellum	-	
Spinal cord		
Dorsal horn	+	Nociception/Analgesia
Ventral horn	+	Spinal reflex

Discussion

The anatomical distribution of the MCH1 receptor in the rat CNS was determined by receptor autoradiography using [³H]Compound 10 at 0.1 nM in the presence of 100 μM dopamine and 1 μM prazosin to directly visualize the receptor (Figure 19 A). Nonspecific binding was determined by including 10 μM unlabeled Compound 10 in the incubation buffer. The specific binding of [³H]Compound 10 was approximately 95% (Figure 19 B).

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The results suggest that the MCH1 receptor is widely distributed in the rat CNS. MCH1 receptors are abundantly expressed in the basal ganglia and moderately expressed in the hippocampus and locus coeruleus. Weak MCH1 expression was observed throughout the diencephalon, mesencephalon and rhombencephalon. The spinal cord exhibited low expression of the MCH1 receptor in the dorsal and ventral horns.

25 MCH-like immunoreactivity (MCH-LI) has been described in the rat CNS (Skofitsch, G. et al. 1985; Zamir, et al., 1986; Bittencourt et al. 1992). MCH-LI was detected

throughout the entire brain, including the neocortex, striatum, amygdala, hippocampus, diencephalon, mesencephalon, and myelencephalon. Only the cerebellar cortex did not contain MCH-LI. MCH cell bodies were located in the hypothalamus, in the olfactory bulb spreading caudally to the anterior amygdaloid area, and in the region of the paramedian pontine reticular formation. The diencephalon contained the highest concentration of MCH-positive cell bodies and an extensive fiber network.

5 Telencephalic areas received a dense MCH immunoreactive fiber network. Sparse MCH positive fibers were seen in the neocortex, hippocampus, olfactory tubercle, caudate-putamen, nucleus accumbens, thalamus, and the medulla and the spinal cord.

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15 Recently with the cloning of the MCH1 receptor (SLC-1) (Saito, et al., 1999; Chambers, et al., 1999) the tissue localization for MCH1 mRNA has been revealed. MCH1 mRNA was localized to a variety of brain regions involved including olfactory regions, the hippocampus, basal ganglia, hypothalamus, amygdala, and locus coeruleus. There was a particularly robust expression of mRNA in the accumbens nucleus which is involved in behavioral reinforcement. Subsequently, using receptor selective 20 antibodies the MCH1 (SLC-1) receptor protein distribution was found to concordant with the distribution of the MCH1 mRNA in the rat CNS (Hervieu, et al., 2000). The distribution of MCH1 binding sites using [³H] Compound 10 herein reported parallels the distribution of both receptor mRNA and protein expression. The extensive distribution of MCH1 receptor binding sites throughout the rat CNS is not surprising because MCH cells in the lateral hypothalamus 25 and zona incerta project widely throughout the brain.

30

Potential Application

MCH has been associated with regulation of food intake and feeding behavior (Qu, et al., 1996; Rossi, et al., 1997; Shimada, et al., 1998), the control of goal oriented behaviors, general arousal or stress responses (Jezova, et al., 1992) and the regulation of fluid homeostasis (for review, Bernardis, et al. 1993).

The anatomical distribution of MCH1 receptor binding sites is consistent with a role for the MCH receptor in the regulation of food intake, thirst, and the reinforcement of feeding behaviors. MCH1 receptor binding sites were evident in the ventromedial, dorsomedial, and arcuate nuclei which are areas that are recognized to be involved in food intake, suggesting that the MCH1 receptor mediates the orexigenic effects of MCH. MCH1 binding sites were present in regions involving the regulation of fluid homeostasis, the lateral hypothalamus and the zona incerta.

As already stated, the MCH1 binding sites are widely distributed throughout the brain. The extensive localization of MCH1 receptors in the neocortex and the lateral hypothalamus supports a functional role for the MCH1 receptor in general arousal.

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MCH has been shown to increase ACTH release *in vivo* and to have a stimulatory effect on the hypothalamic-pituitary-adrenal gland axis (HPA). The site of action of MCH is currently unknown, however one possible target are CRF neurones located throughout the hypothalamus and the bed

nucleus of the stria terminalis. MCH1 receptors have been localized to these regions thus supporting a potential role for the MCH1 receptor in the stress response.

5 MCH1 receptors are present in several limbic system-related structures, namely the hippocampus, septum, accumbens nucleus, nucleus of the diagonal band, bed nucleus of the stria terminalis, and the amygdala. On the basis of this localization, the MCH1 receptor may be involved in the regulation of learning and memory as well as emotional states. It has been established that the drugs that are effective in the treatment of depression and anxiety primarily act on the serotonergic and noradrenergic systems in the brain. MCH1 receptors have been localized in several
10 forebrain areas that receive projections from midbrain raphe nuclei, the origin of the serotonergic pathway, as well as the locus coeruleus where the noradrenergic system originates. MCH1 receptors in the amygdala, hippocampus, hypothalamus, accumbens nucleus and the neocortex may be
15 targets for the treatment of mood disorders.
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The most impressive radioligand binding in the rat CNS was in the basal ganglia, specifically the caudate-putamen, and accumbens nucleus, with moderate binding in the subthalamic nucleus. Taken together with MCH1 receptor localization throughout the motor cortex and the reticular formation, regions associated with locomotion activity, MCH1 receptors may potentially mediate MCH's role in controlling motor behavior and thus may be potential therapeutic target in
25 the treatment of Parkinson's disease and Huntington's Chorea. It is however, noteworthy that there were no
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locomotion deficits found in the open-field locomotion test on MCH^{-/-} mice.

- 5 The localization of MCH1 receptor in the ventral striatum
is rather interesting and suggests that the MCH1 receptor
is a possible therapeutic target in the treatment of drug
addiction and psychosis via the regulation of dopaminergic
neurotransmission. The accumbens nucleus is involved in
the mediation of positive reinforcement of feeding behavior
10 and plays a role in reward mechanisms. There is a dense
dopaminergic projection from the ventral tegmental area to
the accumbens nucleus which is the site of action of
antipsychotic drugs.
- 15 A role for the MCH1 receptor in regulating sensory
information might be indicated by their presence in the
relay nuclei of several sensory pathways. It appears that
the MCH1 receptor may participate in the modulation of the
visual system. MCH1 receptor binding sites are localized
20 to the superior colliculus which receives afferents from
the retina. In the auditory system the MCH1 receptor is
present in the medial geniculate, inferior colliculus, and
the cochlear and medial vestibular nuclei.
- 25 The localization of MCH1 receptors in the locus coeruleus
implies a potential modulatory action in noradrenergic
neurotransmission, influencing sleep, attention and
vigilance.

A potential role for the MCH1 receptor in the modulation of the perception of pain is supported by the localization of MCH1 receptor binding sites in the periaqueductal gray, dorsal raphe nucleus and in the gray matter of the spinal cord. MCH1 receptors are in a position to modulate incoming as well as descending sensory information and also spinal motor reflexes.

In-Vivo Models

Results

1. Effects on MCH-Stimulated Food Intake

The administration of MCH into the third ventricle significantly increased food intake at all time points measured compared to vehicle treated controls (Figure 21). Significant differences in food intake were observed among conditions after 30 [$F(4,44)=7.07$, $p<0.0002$, one-way repeated measure ANOVA], 60 [$F(4,44)=6.31$, $p<0.0004$] and 120 [$F(4,44)=9.84$, $p<0.0001$] min. Pretreatment with systemic DMSO prior to MCH did not significantly alter the MCH-induced food intake (Figure 21). Pretreatment with 10 mg/kg of Compound 10 significantly reduced the magnitude of MCH-induced feeding after 30 and 120 minutes and prevented the occurrence of significant MCH-induced feeding at 60 minutes (Figure 21). Pretreatment with 1 mg/kg of Compound 10 prevented the occurrence of significant MCH-induced feeding over the time period (Figure 21). These results demonstrate that the peripheral administration of Compound 10 significantly attenuates the stimulation of food intake induced by the central administration of MCH.

2. Effects of MCH1 Antagonists on Body Weight

To test the effect of Compound 10 on body weight regulation, we monitored body weight in rats implanted with osmotic minipumps which released either 10 mg/kg/day of Compound 10, 6 mg/kg/day of d-fenfluramine or vehicle. Rats treated with either Compound 10 or d-fenfluramine gained significantly less weight than vehicle-treated rats [by 2-way ANOVA, $F(2,360)=81.69$, $p<0.0001$]. Rats treated with Compound 10 gained significantly less weight than vehicle-

treated controls on days 1-13 (Fig. 22). Rats treated with d-fenfluramine gained significantly less weight than vehicle-treated controls on days 1-6 (Fig. 22). Over the duration of the study, rats treated with Compound 10 or d-fenfluramine gained 16% and 10% less, respectively, from controls.

The effect of Compound 10 on body weight was next tested by administering it to rats twice a day for 7 days by i.p. injection. As shown in Figure 23, while all rats gained weight over the course of the study, rats treated with 10 mg/kg of Compound 10 gained significantly less weight than vehicle-treated controls [effect of treatment by two-way ANOVA: $F(3,214)=60.59$, $P<0.0001$]. Rats treated with 10 mg/kg of Compound 10 gained 26% less weight compared to vehicle-treated rats. There was no significant effect on body weight from the administration of 1 or 3 mg/kg of Compound 10.

The effects of Compound 94 and Compound 95 on body weight were tested by twice daily i.p. administration for 3 days. By 2-way ANOVA, there was a significant effect of treatment on body weight gain [$F(6,160)=31.21$, $p<0.0001$, Figs 24, 5]. As shown in Figure 24, rats treated with 30 mg/kg of Compound 94 gained significantly less weight than vehicle-treated controls on days 1, 2 and 3, while rats treated with 3 or 10 mg/kg of Compound 94 gained weight similarly to controls. Similarly, as shown in Figure 25, rats treated with 30 mg/kg of Compound 95 gained significantly less weight than vehicle-treated controls on days 1, 2 and 3, while rats treated with 3 or 10 mg/kg of Compound 95 gained weight similarly to controls. Over the duration of the

study, rats treated with Compound 94 or Compound 95 gained 40% and 82% less, respectively, from controls. Taken together, these findings demonstrate that three compounds which are antagonists at the MCH1 receptor produce a decrease in body weight gain in young, growing rats.

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3. Effects of MCH1 Antagonists on Consumption of Sweetened Condensed Milk

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Rats were trained to drink sweetened condensed milk during 7 daily 20 min sessions in the early part of the light cycle. Baseline drinking for each animal was determined as the average of the last three days of training. Rats were then exposed to sweetened condensed milk 30 minutes following i.p. administration of either vehicle, 3, 10 or 30 mg/kg of Compound 10 or 3 mg/kg of d-fenfluramine. Figure 26 shows the amount of milk consumed, expressed as a percentage of each animals' baseline drinking. Rats treated with 3, 10 or 30 mg/kg of Compound 10 or 3 mg/kg of d-fenfluramine drank significantly less than vehicle-treated rats (87, 59, 41 and 0.03% less, respectively). These results suggest that Compound 10 acts as an anorectic agent and is capable of decreasing consumption of a palatable food.

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4. Forced Swim Test

The Effect Of Vehicle, Fluoxetine And Compound 10 On Immobility, Climbing And Swimming In The Forced Swim Test

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Immobility

Statistical analysis indicated that there was a significant drug effect [$F(4,24) = 6.36$, $p = 0.0018$] on immobility. Subsequent post hoc analysis revealed that a single injection of 10 mg/kg i.p. of fluoxetine significantly decreased immobility to 23.0 ± 1.0 (Student-Newman-Keuls value was 20.52, $p < 0.01$) compared to vehicle-treated controls (Table 5). In addition, a single injection of either 3, 10 or 30 mg/kg i.p. of Compound 10 significantly decreased immobility (25 ± 2.7 , 25 ± 1.9 & 26 ± 1.9 counts at each dose, respectively) compared to vehicle-treated controls 35 ± 1.9 (Student-Newman-Keuls values of 13.45, 15.08 and 11.91, respectively; Table 5).

Climbing

The statistical analysis of the climbing counts indicated that there was a significant drug effect [$F(4,24) = 5.18$, $p = 0.005$]. Post hoc analysis indicated that a single injection of 10 mg/kg of fluoxetine did not significantly alter climbing counts compared to vehicle-treated animals (Table 5). In contrast, a single injection of 3 mg/kg of Compound 10 produced a significant increase (19 ± 1.7) in climbing counts (Student-Newman-Keuls value = 9.42, $p < 0.05$) compared to vehicle-treated animals (12 ± 1.2). Compound 10 dosed at 10 & 30 mg/kg did not significantly alter climbing.

Swimming

The statistical analysis of the swimming data indicated that there was a significant drug effect [$F(4,24) = 16.4$,
5 $p < 0.0001$] (Table 5). The post hoc test showed that a single injection of 10 mg/kg i.p. of fluoxetine produced a significant increase (26 ± 1.6) in swimming counts over the vehicle treated animals (12 ± 1 ; Student-Newman-Keuls value of 50.48, $p < 0.01$). Similarly, a single injection of 3, 10 or 30 mg/kg i.p. of Compound 10 significantly increased
10 swimming counts (16 ± 1.1 , 20 ± 1.7 & 23 ± 1.0 , respectively; Student-Newman-Keuls values of 4.37, $p < 0.05$, 19.26, $p < 0.01$; 34.2, $p < 0.01$; Table 5).

Diving

15 This behavior was not observed following a single injection of vehicle and rarely observed following fluoxetine (1 animal out of 5 dove twice), 3 mg/kg of Compound 10 (2 animals out of 5 dove once each), 10 mg/kg of Compound 10 (4 animals out of 5 had counts of 1, 4, 1 and 3) or 30 mg/kg of Compound 10 (2 rats out of 5 had counts of 1 and 20 4).

TABLE 5. The effect of vehicle, Compound 10 and fluoxetine on immobility, climbing and swimming in the rat forced swim test.

5	Treatment	Dose (mg/kg)	Immobility	Climbing	Swimming
	Vehicle	-----	35 ± 1.9 ^c	12 ± 1.2	12 ± 1.0 ^a
10	Compound 10	3	25 ± 2.7	19 ± 1.7 ^b	16 ± 1.1
	Compound 10	10	25 ± 1.9	13 ± 1.7	20 ± 1.7
	Compound 10	30	26 ± 1.9	10 ± 1.8	23 ± 1.2
15	Fluoxetine	10	23 ± 1.0	12 ± 0.8	26 ± 1.6

Each value represents the mean ± S.E.M. A total of 5 animals were examined for each treatment group.

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^aSignificantly less than 3 mg/kg Compound 10 ($p < 0.05$) and 10 and 30 mg/kg Compound 10 and fluoxetine, ANOVA and Student-Newman-Keuls test.

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^bSignificantly greater than Vehicle ($p < 0.05$) and 10 and 30 mg/kg Compound 10 ($p < 0.01$), ANOVA and Student-Newman-Keuls test.

^cSignificantly greater than 3, 10 and 30 mg/kg Compound 10 and fluoxetine, $p < 0.01$, ANOVA and Student-Newman-Keuls test

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The results of the Forced Swim Test indicate that using a modified version of the Porsolt forced swim test, a single injection of 10 mg/kg i.p. of fluoxetine produced a significant decrease in immobility and an increase in swimming in male Sprague-Dawley rats. This is consistent with findings from previous studies using the Lucki version (Detke, et al., 1995; Kirby and Lucki, 1997; Lucki, 1997; Page, et al., 1999; Reneric and Lucki, 1998). In addition, the results obtained using fluoxetine are consistent with those using other SSRIs (Detke, et al., 1995). Thus, a modified version of the Porsolt forced swim test can consistently detect the antidepressant action of SSRIs such as fluoxetine.

Compared to vehicle-treated animals, Compound 10 produced a significant decrease in immobility and a significant increase in swimming at doses of 3, 10 and 30 mg/kg, and a significant increase in climbing at 3 mg/kg. Thus, based on past interpretations of the Forced Swim Test, our results suggest that Compound 10 has antidepressant-like properties.

5. Social Interaction Test

The Effect Of Compound 10 And Chlordiazepoxide On Behavior In The Rat Social Interaction Test

A single i.p. administration of either 3, 10 or 30 mg/kg of Compound 10 significantly increased social interaction (Student-Newman-Keuls values of 48.7, 43.7 & 17.1, respectively), as did the benzodiazepine anxiolytic,

chlordiazepoxide (Student-Newman-Keuls value of 58.8) compared to vehicle-treated animals [ANOVA, $F(4,40) = 20.6$,
p < 0.0001; Table 6]. The degree of social interaction produced by the 30 mg/kg i.p. dose of Compound 10 was
5 significantly less than that of 3 and 10 mg/kg of Compound 10 and 5 mg/kg of chlordiazepoxide (Student-Newman-Keuls values of 8.06, 8.12 and 14.16, respectively). There was no significant difference in the duration of social interaction between 3 and 10 mg/kg i.p. of Compound 10 and
10 chlordiazepoxide.

Table 6. The Effect Of A Single Injection Of Vehicle,
Chlordiazepoxide And Compound 10 On The Social Interaction
And Rearing Of Unfamiliar Cage Mates In A Familiar Arena

5 Drug Treatment Dose Social Interaction (sec)

10	Vehicle	-----	102 ± 6.1 ^A
	Chlordiazepoxide	5 mg/kg	228 ± 11*
	Compound 10	3 mg/kg	210 ± 14*
	Compound 10	10 mg/kg	215 ± 15*
	Compound 10	30 mg/kg	166 ± 11*&

15 ^AEach value represents the mean seconds of social interaction ± S.E.M. A total of 7-10 animals were examined for each treatment group.

20 *Significantly greater than Vehicle, $p < 0.01$, ANOVA and Student-Newman-Keuls test.

25 ^aSignificantly less than chlordiazepoxide, 3 mg/kg of Compound 10 ($p < 0.01$) and 10 mg/kg of Compound 10 ($p < 0.05$), ANOVA and Student-Newman-Keuls test.

The Effect Of Compound 10 And Chlordiazepoxide On Rearing Behavior, Locomotor Activity And Grooming In The Rat Social Interaction Test

5 Statistical analysis indicated a significant effect of treatment on rearing behavior (ANOVA, $F(4,40) = 3.03$, $p = 0.028$, Table 7). A single i.p. administration of 3 mg/kg of Compound 10 significantly lowered the number of rearings displayed compared to animals treated with vehicle and 10 or 30 mg/kg of Compound 10 (Student-Newman-Keuls values of 10 4.55, 5.34 and 8.09, respectively). In addition, the number of rearings produced by 5 mg/kg i.p. of chlordiazepoxide was significantly lower than that for the 30 mg/kg dose of Compound 10 (Table 7).

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Statistical analysis indicated a significant effect of treatment on grooming behavior ($F(4,40) = 12.00$, $p < 0.0001$; Table 7). A single i.p. administration of 30 mg/kg of Compound 10 produced a significantly greater number of grooming bouts compared to animals treated with vehicle, 3 or 10mg/kg of Compound 10 and 5 mg/kg of chlordiazepoxide (Student-Newman-Keuls values of 16.9, 25.1, 27.9 and 36.6, respectively). There was no significant difference in the number of grooming bouts between the 3 and 10 mg/kg doses of SNEC-3 and vehicle-treated animals.

30 Statistical analysis indicated a significant effect of treatment on locomotor activity ($F(4,40) = 3.93$, $p = 0.0088$). Post hoc analyses indicated that the number of squares crossed following the 3 mg/kg dose of Compound 10 was significantly lower than animals treated with either

vehicle, 10 or 30 mg/kg of Compound 10 (Student-Newman-Keuls values of 8.4, 6.5 and 8.96, respectively, Table 7). There was no significant difference in the number of squares crossed between animals treated with a single injection of 5 mg/kg of chlordiazepoxide and the other treatment groups.

Table 7. The effect of a single injection of vehicle, chlordiazepoxide and Compound 10 on the number of rearings, grooming episodes and squares crossed in the social interaction test.

Drug Treatment	Dose	Rearing	Squares Crossed	Grooming Bouts
Vehicle	-----	56 ± 6	484 ± 52	6.2 ± 0.7
Chlordiazepoxide	5 mg/kg	46 ± 5	355 ± 49	3.6 ± 0.7
Compound 10	3 mg/kg	43 ± 4 ^a	327 ± 24 ^c	5.2 ± 0.5
Compound 10	10 mg/kg	59 ± 3	480 ± 46	4.3 ± 0.4
Compound 10	30 mg/kg	61 ± 4 ^b	490 ± 29	10.8 ± 1.3 ^d

All values represent the mean \pm S.E.M. A total of 7-10 animals were examined for each treatment group.

^a Significantly less than vehicle, 10 and 30 mg/kg Compound 10, $p < 0.05$, ANOVA and Student-Newman-Keuls test.

^b Significantly greater than chlordiazepoxide ($p < 0.05$) and 3 mg/kg of Compound 10 ($p < 0.01$), ANOVA and Student-Newman-Keuls test

^c Significantly less than vehicle, 3 and 30 mg/kg Compound 10 ($p < 0.05$), ANOVA and Student-Newman-Keuls test.

25 ^d Significantly greater than all other treatment groups, p
 < 0.01, ANOVA and Student-Newman-Keuls

At doses of 3, 10 and 30 mg/kg i.p., Compound 10 produced a significant increase in social interaction time in male rats compared to vehicle-treated animals. Also, the anxiolytic agent (5 mg/kg i.p. chlordiazepoxide) produced a significant increase in social interaction time compared to vehicle-treated animals. The response produced by either the 3 or 10 mg/kg doses of Compound 10 was comparable to that of the positive control, chlordiazepoxide. The degree of social interaction produced by the 30 mg/kg i.p. dose of Compound 10 was significantly lower than that for the 3 and 10 mg/kg doses. Since this higher dose of Compound 10 resulted in a significant increase in grooming behavior compared to other treatment groups, it is possible that it was producing behaviors that were competing with the social interaction time.

A single administration of 3 mg/kg of Compound 10 produced a significant decrease in the number of squares crossed and rearing episodes compared to animals treated with vehicle and 10 or 30 mg/kg of Compound 10. The behavioral significance of this finding is unknown. However, the animals treated with the 3 mg/kg dose of Compound 10 did not show any obvious signs of catalepsy or sedation. In addition, the level of social interaction at this dose was not significantly different from that of 10 mg/kg of Compound 10 or 5 mg/kg of chlordiazepoxide.

Previously, it has been shown that in the social interaction test, analeptics such as amphetamine and caffeine, increase social interaction and locomotor

activity, whereas anxiolytics increase social interaction time but actually decrease locomotor activity (File, 1985; File and Hyde, 1979; Guy and Gardner, 1985). However, none of the doses of Compound 5 10 significantly increased either rearing behavior or squares crossed compared to vehicle-treated animals. In fact, as stated above, the number of squares crossed and rearing behavior was significantly reduced at the 3 mg/kg i.p. dose of Compound 10 compared to vehicle-10 treated animals. Thus, it is unlikely that Compound 10 is producing a non-specific effect.

In conclusion, the results of this study indicate that Compound 10, at doses of 3, 10 and 30 mg/kg i.p., 15 significantly increased social interaction time without producing a significant increase in squares crossed or rearing behavior. Overall, Compound 10 appears to have the profile of an anxiolytic drug in the social interaction test.

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